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A CONTRIBUTION TO THE BIOLOGY OF FRUIT-FLY PARASITES IN HAWAII

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INTRODUCTION

With the termination of an intensive study of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) in Hawaii in February, 1916, by the Bureau of Entomology, a general investigation of the biology, interrelation [1] and economic value of introduced parasites of this fruit fly was begun. The results of this investigation are herewith presented.

The natural enemies of the Mediterranean fruit fly now contributing toward its control in Hawaii are three species of Opiinae—viz, *Diachasma tryoni* Cameron, *Opius humilis* Silvestri, and *D. fullawayi* Silvestri, one species of Eulophinae, *Tetrastichus giffardianus* Silvestri, and an ant, *Pheidole megacephala* Fabricius. Two other parasites occasionally reared from the fruit fly are *Opius fletcheri* Silvestri, normally a melon-fly parasite, and *Pachycrepoideus dubius*, a dung-fly parasite. At present parasitism by these two parasites is not important, and may be more accidental than normal. The opiines and the eulophid are strictly larval parasites. The ant is important as a predacious enemy of the larvæ and to a lesser extent of the pupæ. The pteromalid *Pachycrepoideus dubius* attacks the pupa. There is striking similarity in habit, structure, and development among the opiines under discussion, and in view of this the species now most abundant in Hawaii, *Diachasma tryoni*, will be dealt with the most completely, to be followed by notes on the special features of the two other important opiine species together with comparisons with the species *tryoni* in sufficient detail to give a thorough conception of the biology of each.[2]

[1] For previous studies on fruit-fly interrelations in Hawaii, see Pemberton and Willard (6). [Reference is made by number (italic) to "Literature cited," p. 465.]

[2] For the original descriptions and history of the introductions of the important species above mentioned, see BACK, E. A., and PEMBERTON, C. E. (3).

For detailed records on the extent of parasitism in Hawaii by these species see Back and Pemberton (1, 2) and Pemberton and Willard (5).

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DIACHASMA TRYONI

Diachasma tryoni Cameron was first observed in New South Wales in 1908 and was described in 1911. It was introduced into Hawaii from New South Wales by Silvestri in May, 1913. It soon became definitely established and by 1916 its importance as a parasite and its ready adaptation to Hawaiian conditions were demonstrated clearly. During 1917 it excelled the work of the other introduced parasites

DESCRIPTION AND LIFE HISTORY

EGG

The egg (fig. 1, 2) is cylindrical, translucent white, with smooth, glistening surface, drawn out at each end into a short, rounded protuberance and when first deposited is surrounded by a thin, transparent membrane, possibly the exochorion. This membrane adheres to the conformity of the egg but does not tightly inclose it. The egg is faintly concave ventrally and distinctly convex dorsally. The protuberance on the cephalic end is slightly broader and shorter than is that on the caudal end. At deposition the egg averages 0.45 mm. in length, including the enveloping membrane, its greatest width being about one-sixth of the length. When fully developed the egg averages 0.65 mm. in length and is about one-fourth as broad as long. During development the outer enveloping membrane is ruptured and entirely separated from the egg proper. At maturity each end of the egg stands out as an abrupt, broad tubercle. The embryo is then clearly revealed by transmitted light.

Fig. 1.—*Diachasma tryoni:* Egg just laid; length 0.48 mm.

Although the eggs are deposited only slightly beneath the surface of the larval skin, they are invisible even under strong sunlight and magnification. The duration of the egg period can be determined only by dissections of host tissue at frequent intervals to locate the eggs and by the use of numbers of well-parasitized larvæ known to have been oviposited in for a short and definite period. The egg stage in Honolulu (Table I) was found to last from 54 to 73 hours, the variations depending upon fluctuations in temperature. As the average incubation period is about 2½ days, the exact number of hours required for the development of eggs deposited in the morning is less than is that where eggs are deposited in the late afternoon. Eggs deposited in the morning hours occupy parts of three days and two nights before hatching, and thus develop under a somewhat higher average temperature than would obtain in the case of eggs deposited in the afternoon or early evening.

Fig. 2.—*Diachasma tryoni:* Egg mature; length 0.65 mm.

These pass through parts of three nights and two days as opposed to three days and two nights and would thus be subjected to somewhat lower average temperatures. This will explain some of the misleading variations shown in Table I.

TABLE I.—*Duration of the egg stage of Diachasma tryoni in Honolulu*

Number of eggs under observation.	Eggs deposited.	Eggs hatched.	Average duration of egg stage.	Mean temperature.
			Hours.	°*F.*
24.....	Jan. 4, 11 a. m. to 1 p. m...	Jan. 7, 11 a. m. to 2 p. m...	73	71. 0
138.....	Mar. 21, 11 a. m. to 1 p. m	Mar. 24, 9 a. m. to 10 a. m.	70	73. 2
130.....	Apr. 16, 9 a. m. to 10 a. m.	Apr. 18, 10 p. m. to 11.30 p. m.	62	74. 2
73.....	May 31, 3 p. m. to 3. 30 p.m.	June 3, 6 a. m. to 8 a. m...	64	74. 0
189.....	July 6, 11 a. m. to 2 p. m..	July 8, 6 p. m. to 10 p. m..	56	75. 9
38.....	July 7, 2 p. m.....	July 9, 9 p. m. to 11 p. m..	56	76. 0
47.....	Oct. 5, 8 a. m. to 10 a. m..	Oct. 7, 1 p. m. to 4 p. m....	54	76. 6

The fully developed egg is so swollen and the membrane so thin that actual emergence of the larva from the egg is sudden, and rather an explosive process. Many eggs on hatching have been under observation. The egg membrane is suddenly ruptured longitudinally, probably by the movements of the larva within, and the larva floats out without apparent effort into the semiliquid medium of the host surrounding it. The egg may hatch while the host is still in the active, feeding, larval stage or it may hatch after the puparium is formed and the complete histolysis of larval tissue has taken place. No important histogenetic action occurs in a puparium containing a parasite egg or larva. The parasitized host larva feeds and develops to maturity even though heavily superparasitized, leaves the fruit normally, and forms a perfect puparium in the usual manner. The complete histolysis of the larval tissues within the puparium then follows, but here all fly development ceases. Henceforth the content of the puparium is but a liquid mass containing the broken-down bits of larval tissue and the rapidly developing parasite larva. The death of the host then may be said properly to occur at the cessation of histolysis in the newly formed puparium. From over 3,000 parasitized puparia opened during 1916 and 1917 no single case was ever noted in which a perfect or even partially formed fly pupa occurred.

LARVA

The larva undergoes many interesting phases in the processes of transformation. When first hatched, it is about 0.85 mm. long. This is the most active period in the larval life. At this time it is so markedly different from the later instars that it appears to simulate the larval structure and habits of an ancestral type. It usually hatches while the host is still in the larval stage. The parasitic larva then lies in a well-

organized body, wherein its food, which seems largely the fat body of the host, is in a semisolid state, in part isolated into definitely compacted masses. The larva is lodged in an area which is well organized in muscular, digestive, nervous, and respiratory structures, all of which combine to interfere with its freedom of action. Special characters, not appearing in the succeeding instars, are peculiarly adapted to this stage. The head is large, heavily chitinized and brownish, and bears a pair of sickle-like mandibles, with bases broadly separated and capable of wide movement and quick action. Above the mandibles and seemingly on the labrum is a pair of small, short antennal structures, which are frequently extended and withdrawn in a rapid, vibratory manner as the larva feeds and moves about. On the cephalic edge of the chitinized ventral portion of the head is borne a pair of pointed teeth, well separated and together forming a distinct letter U with the basal connecting line more or less straight. This lies below and directly between the bases of the mandibles (fig. 3). Its shape affords the best character for differentiating the larva of this species from the newly hatched larva of *Diachasma fullawayi* or that of *Opius humilis*. A clearly defined, simple tracheal system is present (fig. 4) and becomes filled with air shortly after the larva has hatched. No spiracles occur, but eight minute, oval swellings can be seen along each main tracheal trunk in body segments 1 to 8. The larva lies strictly within the host, and the air which quickly fills the tracheæ must be obtained by osmosis from the aerated liquid media surrounding it. The tracheæ are filled with air before food has been taken, which shows that the air is not extracted internally from the ingested food. The digestive canal (fig. 5) is a simple, straight tube

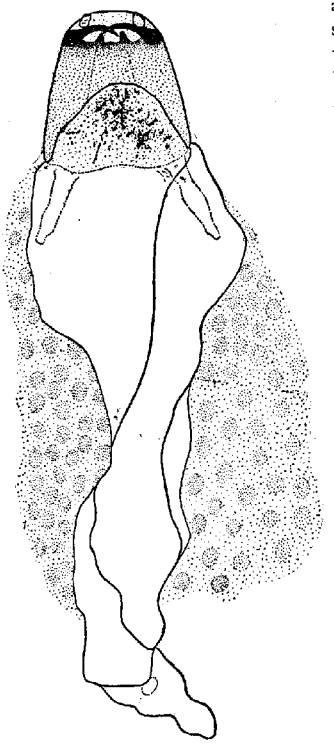

FIG. 3.—*Diachasma tryoni*: Cast skin of first-instar larva, showing head characters of first instar and serosal cellular mass still clinging to ventral surface. Length 1 mm.

with short, narrow esophagus, large midintestine occupying the greater bulk of the body and closed caudally, and the short proctodæum terminating with an apparently open, oval anus situated on the ventral surface of the third to the last body segment. The only food taken that is readily visible is the fat of the host. With the development of this instar the midintestine becomes gradually filled and swollen with globules of fat. Newly hatched larvæ generally are found moving about in the fat body and have been dissected frequently from fly larvæ with the mandibles closed into portions of the fat. Though the large, pointed mandibles enable the larva to lacerate tissues other than the fat body, through some unknown influence the delicate vital organs of the host larva seem never to be injured, even in cases of superparasitism when six or eight newly hatched larvæ may be cutting about with their mandibles, either in the separation of food or in the destruction of one another. The first-instar larva moves about by contorting the body, and its movements are aided by gripping fresh tissues coming into contact with the mandibles coincident with the body movements. The brownish, chitinized head can be seen moving within the host larva when under strong light and fair enlargement. The larva is legless, but bears a pair of soft, short, saclike appendages on the ventral side of the body just back of the head (fig. 3, 4). They are incapable of movement and may be gill-like in their function. No tracheæ can be seen leading into them even when examined under high magnification. Extending along the ventral surface of the body, from the back of the head to the tip of the abdomen, is a gelatinous mass of large cells. These are the serosal cells of the egg and adhere to the larva until it molts for the first time (fig. 3). Just before molting the larva becomes greatly engorged with food and has increased to about 1.2 mm. in length.

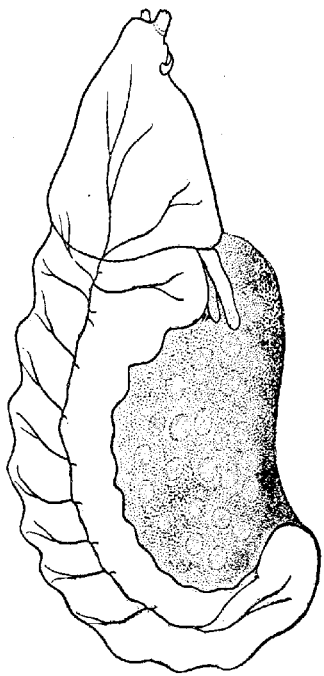

FIG. 4.—*Diachasma tryoni*: Larva of first instar, lateral aspect, showing right main tracheal trunk with branches, and characteristic position and volume of egg serosal cells clinging to ventral surface. Length 1 mm.

The duration of the first-instar larva is dependent upon a curious circumstance. The larva never molts until the fruit-fly larva attempts to pupate. Thus, a small fly larva may be stung by the parasite while the larva is in a fruit of dry texture or hard pulp. Usually the larva will not mature or try to pupate until from 6 to 10 days later when in such a fruit. In this case the parasite egg hatches in the usual time (54 to 73 hours) and the parasitic larva remains in the first instar during the long 6- or 7-day period remaining until the host prepares to pupate. Under ordinary circumstances the parasite stings a mature larva, which usually forms a puparium a few days later. Here the parasitic larva hatches about the time the host larva is fully developed and ready to attempt pupation. The first instar in such cases lasts from 36 to 48 hours. The following specific cases were observed during 1917: Two small fruit-fly larvæ, each stung by a female of *Diachasma tryoni* on June 14, still contained first-instar *tryoni* larvæ on June 21. Thirty-one small fruit-fly larvæ, stung by *tryoni* females on August 1, still contained first-instar *tryoni* larvæ when opened on August 8. On August 12 four had formed into puparia by 9 a. m.[1] They were opened on the same day at 4 p. m. and each was found to contain a freshly transformed second-instar *tryoni* larva. The other fly larvæ did not show signs of pupating after several days. Four of them were opened on August 16 and each contained a first-instar *tryoni* larva. The remaining four, still active, were opened August 18 and found to contain a well-developed *tryoni* larva in the first instar. In this series of examinations of the 12 parasitized fly larvæ the first 4 produced *tryoni* larvæ whose first instar lasted about 36 hours, the second 4 contained *tryoni* larvæ whose first instar had already lasted about 6 days and the last 4 contained *tryoni* larvæ whose first stage had already extended about 8 days. The extended time in the duration of the instar was in each case entirely controlled by the delay of the host in attempting to pupate.

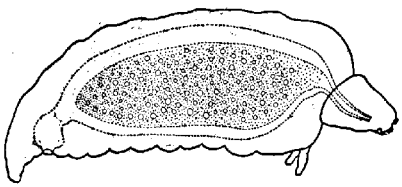

FIG. 5.—*Diachasma tryoni*: Larva of first instar about to molt, lateral aspect showing food canal filled with fat globules and illustrating the beginning of the formation of the meconium. Length 1.5 mm.

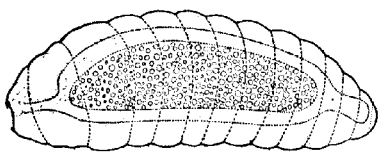

FIG. 6.—*Diachasma tryoni*: Larva in second instar, dorsal aspect, showing general shape of body and food canal. Length 1.5 mm.

[1] All references to clock time refer to "standard time."

When the larva has molted to the second instar, the molted skin can be dissected easily from the fly puparium (fig. 3). In the second instar the larva is greatly changed (fig. 6). The head does not stand out strongly differentiated from the other body segments as in the preceding instar. It is soft, unchitinized, and without pronounced visible characters. The articulations of the 14 body segments can be clearly seen. The body is glabrous throughout. The mandibles (fig. 7) are soft and translucent and can be seen only with difficulty. The weight of a coverglass may easily crush them beyond recognition. They are sharply pointed, short, and about as long as broad, averaging 0.021 mm. in length. Mandibles are not needed in this instar, as the food is composed entirely of fluids, minute globules of fat, and possibly fragments of disintegrated tissue. As the development of the larva progresses the mandibles of the third instar may be seen distinctly pushing into ultimate position at the bases of the mandibles. The larva averages about 1.5 mm. in length in this stage.

FIG. 7.—*Diachasma tryoni:* Mandible of second-instar larva, showing mandible of third instar pushing from within. Length 0.021 mm.

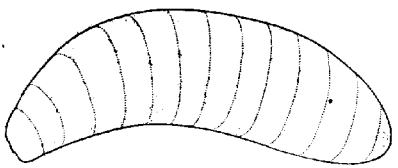

FIG. 8.—*Diachasma tryoni:* Larva of the third instar, dorsal aspect. Length 2.9 mm.

One striking feature in the second instar is the total absence of tracheæ, as careful examinations of more than 100 second-instar larvæ under the highest magnification and best light failed to reveal any evidence of tracheal trunks or branches. In view of the presence of a well-marked respiratory system in the preceding instar, the absence, at this stage, of tracheæ is of distinct interest. As the larva is now immersed in a thin liquid there would seem to be no need for tracheæ. The digestive tube is filled with food, and, as in the first instar, takes the form of the simple midintestine. The oily fat globules of the host which are ingested are conspicuous in this portion of the intestine. This intestine is closed caudally, although the short hind intestine may be seen leading up to it. In this instar the larva is very sluggish, and there is no need for action, considering the accessibility and character of the food. The duration of this stage has not been determined accurately. There is no wide variation in its length, however, such as occurs in the first and fourth instars. The average duration of the second larval instar is about 48 hours. There

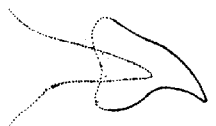

FIG. 9.—*Diachasma tryoni:* Mandible of third-instar larva, showing mandible of fourth instar pushing from within. Length 0.035 mm.

is little to distinguish the third (fig. 8) from the second larval instar, and it is even less pronounced in character. The mandibles (fig. 9) are slightly heavier and are about 0.035 mm. in length. Otherwise they are almost identical with those of the previous instar. The mandibles of the forming fourth instar can be seen pushing from within into the bases of the mandibles. There are no hard, darkened or chitinized parts in any portion of the body. As in the preceding instar, no traces of tracheæ occur, but late in the development of this stage the strong, heavy tracheal trunks, branches, and stigmata of the succeeding instar may be seen organizing beneath the skin. The stigmata do not, at any period in the development of the third instar, become opened to the surface, as they are not a developed accessory of this stage, but belong strictly to the succeeding instar. The body is glabrous throughout. Late in the development of this stage the spiny cuticula of the succeeding instar may be seen beneath the integument. No change has been noted in the digestive tract, other than that of a gradual increase in the volume of food ingested and the increased volume of waste matter accumulating in the closed midintestine (fig. 10). This stage averages about 2.4 mm. in length. It still lies immersed in the body fluids of its host, although shortly before molting to the last instar a large part of the body is usually exposed in the hollow puparium. The average duration of the third larval instar will approximate 48 hours.

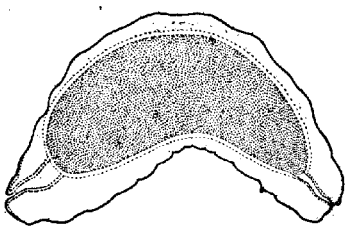

FIG. 10.—*Diachasma tryoni*: Third-instar larva, lateral aspect, showing digestive canal. Length 3.1 mm.

When the fourth instar is reached, the conditions surrounding the larva have undergone a great change. Much of the liquid and semiliquid contents of the host have been consumed, and within a short time little remains in the puparium but the parasitic larva. To meet this condition, the parasitic larva is possessed from the first of a well-defined tracheal system. Nine large, open stigmata are borne on each side of the second to the eleventh body segments (fig. 11). No stigmata occur on the third segment, however, although a branch from the tracheal trunk on each side leads to points on the surface corresponding in position with the stigmata on the other body segments. A main tracheal trunk extends along each side of the body with a special branch to each stigma and to the dorsal and ventral portion of each body segment. A connecting branch joins the two main trunks posteriorly and anteriorly. With the exception of the head the entire body is closely covered with

short, sharp, curved spines with broad bases (fig. 12, 13). These spines are absent along the line of articulation between each segment. The body averages 3.1 mm. in length and about 1 mm. in width. The characters of the head are strongly developed (fig. 14). A well-defined labrum, heavily pointed mandibles about 0.12 mm. long, with broad rounded bases and brownish chitinization at the tips and bases (fig. 15), distinct maxillæ with short major and minor palpi, and a chitinized labium with palpi, can be distinguished readily. The palpi are only short tubercles. Well-developed, yellowish, tentorial ridges in the head support the mouth parts. The mandibles and tentorial structures of the head are colorless at first. Some hours after the molt they assume the yellowish-brown color that so readily distinguishes this instar from the preceding. The head is about 0.5 mm. in width. As the remaining food consumed by this instar is liquid, the purpose of the well-developed mandibles, which may be vigorously moved, has not as yet been established. No change takes place in the alimentary canal upon the molt to this instar. The larva is very sluggish, though it may bend its body slowly from side to side. It usually lies with its head in the head end of the puparium. Of 76 parasitized fruit-fly puparia opened to determine this point, 64 contained mature *Diachasma tryoni* larvæ in this position. In the remaining 12 the position was reversed. When in the first instar, the larva moves about with its head in no constant direction.

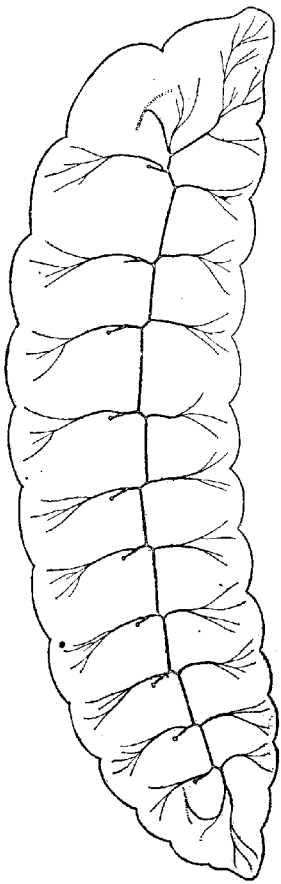

Fig. 11.—*Diachasma tryoni:* Larva of fourth instar, lateral aspect, showing complete right tracheal trunk with branches and stigmata. Length 3.1 mm.

The duration of the mature larval stage is of unusual interest. In Hawaii it may extend from about 6 days to over a year. Larvæ ordi-

narily pupate within 8 or 9 days after the host puparium is formed and the adult parasites emerge in from 5 to 8 days later. A certain proportion of the larvæ reaching maturity each month of the year, however, pass into a dormant state and may remain in this condition for from a few weeks to several months, or occasionally a year. No doubt periods of long drouth and scarcity of host material in Australia, in the localities

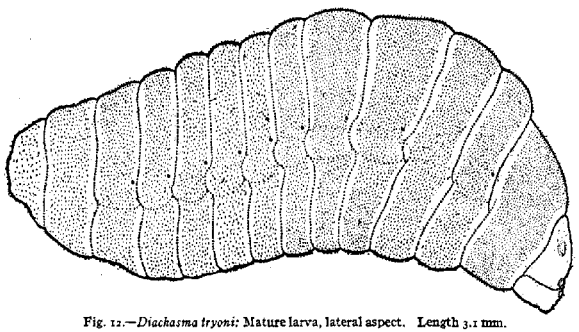

Fig. 12.—*Diachasma tryoni:* Mature larva, lateral aspect. Length 3.1 mm.

where this species is probably native, are frequent, and these conditions may have necessitated such an adaptation. This parasite may thus persist under very unfavorable conditions. With its introduction into Hawaii this strong, inherent trait endures, even though climatic and host conditions are ideal for continuous reproduction throughout the year. The hibernating larvæ look much the same as do other mature larvæ, although the body is somewhat contracted, shortened, and slightly paler in color. During the entire period of dormancy the larva is capable of slow movement. The dormant larvæ are not necessarily undernourished individuals. In fact, hibernation has been noted among individuals possessed of large, fully nourished bodies. No great variation in the degree of hibernation has been noted to occur in fly larvæ from any special variety of fruit. The number of males produced from hibernating material greatly exceeds the number of females. Between August, 1916, and July, 1917, 663 males to 118 females emerged from hibernating lots, ranging from 1 to 12 months old.

Fig. 13.—*Diachasma tryoni:* Greatly enlarged view of spines covering surface of body of mature larva. Length, 0.01 mm.

Parasitized fruit-fly puparia placed in dry glass vials or jars yield a much lower percentage in hibernation than do puparia left in the soil

under natural conditions. Thus, in March, 1917, a total of 2,725 French cherries, *Eugenia uniflora*, collected in Honolulu, yielded 1,213 puparia parasitized by either *Diachasma tryoni* or *D. fullawayi*. One-half of the puparia were placed in glass vials and the remainder in a screened box and covered with ½ inch of sand. Of the lot placed in vials 242, or 39.9 per cent, of the larvæ of *Diachasma* spp. hibernated, and of those in the sand 543, or 89.4 per cent, entered hibernation. Again, in September, 1917, during a warm part of the year, a quantity of kamani nuts (*Terminalia catappa*) was collected in Honolulu and placed in a large screened box containing sand, and left in the open air. Of 785 parasitized fruit-fly puparia forming in the sand in this box 271 produced living adults of *D. tryoni* in the usual time, while the remaining 514, or 65.5 per cent, upon examination late in October were found to contain living larvæ of *D. tryoni*. A repetition of this experiment was started in November, 1917. Of 934 parasitized fruit-fly puparia forming in the sand in the box from November 2 to 20, only 69 produced adults of *D. tryoni* in the usual time. The remaining 865 puparia were opened in January, 1918, and all were found to contain living larvæ of this species. This is an average hibernation of 92.6 per cent and repre-

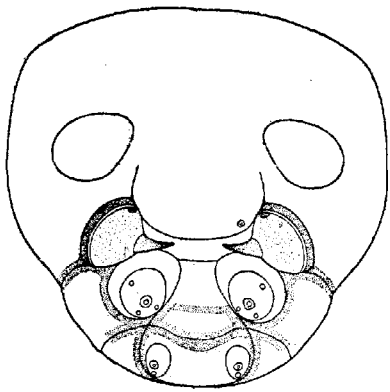

Fig. 14.—*Diachasma tryoni:* Head of mature larva, dorso-cephalic view. Greatest width 0.50 mm.

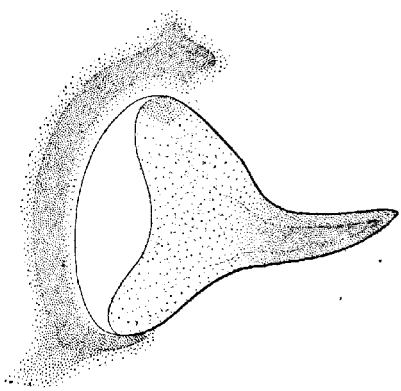

Fig. 15.—*Diachasma tryoni:* Mandible of mature larva. Length 0.12 mm.

sents fairly natural conditions. Table II shows the extent of hibernation occurring in the laboratory among fruit-fly puparia that were collected during every month throughout the year. The greatest hibernation occurred during the winter months commencing in December and the least during the warmest months. As the data are secured from material held in the laboratory in glass, the degree of hibernation is lower than would obtain in the field normally, which is evidenced by the box experiments.

TABLE II.—*Hibernation of Diachasma tryoni and D. fullawayi*[a]

Host puparia collected.	Total number of parasitized puparia.	Number of *D. tryoni* emerging in normal time.	Number of *D. fullawayi* emerging in normal time.	Number of Diachasma larvæ going into hibernation.	Total percentage of hibernation.
1916.					
December	302	193	10	99	32.8
1917.					
January	1,816	791	83	942	51.9
February	774	367	120	287	37.1
March	1,599	765	331	503	31.5
April	1,055	882	136	37	3.5
May	1,942	1,767	153	22	1.1
June	925	902	17	6	0.6
July	1,263	1,218	41	4	0.3
August	1,605	1,356	236	13	0.8
September	2,946	2,821	81	44	1.5
October	1,960	1,558	374	28	1.4
November	2,069	1,692	285	92	4.4
December	1,116	744	189	183	16.4

[a] The ratio of hibernating larvæ of *Diachasma tryoni* is greater than is that of *D. fullawayi*, but just how much greater as yet has not been determined.

The greatest emergences of adults from hibernating individuals occurs during the first seven months after the larva goes into hibernation. From 781 larvæ going into hibernation between August 1, 1916, and July 1, 1917, inclusive, 129, 119, 36, 67, 128, 147, 81, 27, 19, 16, 9, and 3 pupated and became adult during the period from the first to the twelfth month, respectively. As the greater number began their dormant period during the winter months and as the average duration of this period is from two to six months, it follows that the greatest emergence from hibernating individuals occurs in the spring and early summer months in Hawaii.

No doubt more than one factor enters into the cause of hibernation among the larvæ of the two species of Diachasma. Cool temperatures seem the most important, as suggested in Table II. During August, September, and October of 1917 the total hibernation, as occurring in material placed in glass vials, was 0.8, 1.5, and 1.4 per cent, respectively, based upon records of 1,605, 2,946, and 1,960 parasitized puparia, respectively. The average mean temperature at which this material was kept during the three months was 78.7°, 74.7°, and 75° F., respectively.

In the same months a quantity of parasitized, freshly formed fruit-fly puparia was placed in similar glass vials in a refrigerator running evenly from 60° to 64° during the entire duration of the experiment. From this material 384 adults of species of Diachasma emerged over a period somewhat retarded but not greatly prolonged, while 814 puparia failed to produce anything and upon being opened on December 20 were found to contain living, hibernating larvæ of this genus. This represents a hibernation of 67.7 per cent of all the material parasitized by species of Diachasma that was placed in the refrigerator, and is striking when compared with the hibernation of slightly over 1 per cent among the larvæ of this genus held at the same time at a temperature about 13 degrees higher. Another unknown cause for hibernation must exist, as material kept beneath sand or soil at any time of the year produces a greater degree of hibernation than does material held coincidently at nearly the same temperature but in dry glass vials exposed to light.

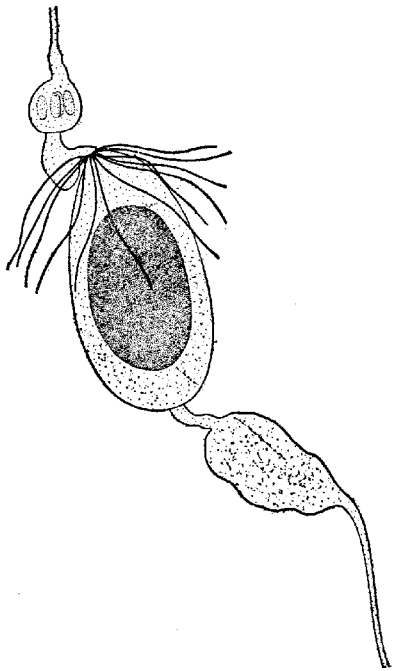

FIG. 16.—*Diachasma tryoni*: Alimentary canal removed from a mature pupa, showing the position and shape of the meconium. Greatly enlarged.

PUPA

The pupa is from 3.5 to 4 mm. long by 1.6 mm. wide and at first is pale white with reddish eyes. In a few days the adult colorations appear. At pupation the old larval skin is split from the head backwards and slips back to the caudal tip of the pupa and is there immediately pushed forward by the tips of the antennæ of the male or ovipositor of the female as these parts are forced forward over the dorsum of the body. The exuvium then comes to rest as a yellowish, crumpled mass at the tip of the ovipositor at a point generally over the pupal metathorax and extending, in part, back along the side of the ovipositor, or,

in the case of the male, it lies at the tips of the antennæ over the first two abdominal segments. When the adult parasite emerges, this exuvium is carried about for a short time on the antennæ of the male or the ovipositor of the female. With the complex changes in the alimentary canal, accompanying the formation of the pupa, the unvoided waste and accumulated food which fills the larval midintestine is forced to occupy a greatly reduced space. In the pupa, then, the midintestine is found to be short, oval, and filled with a dark compressed pellet (fig. 16). This pellet is the meconium. No portion of it is voided at the time of pupation or during the pupal period. In the mature pupa this meconium occasionally may pass into the hind intestine just caudad of the urinary tubes, but it never passes from the pupa. The pupa stage, following a short prepupal period of from 1 to 2 days, may last from 6 to 10 days. The duration of the pupa stage varies at any time of year.

The duration of the combined egg, larval, and pupal periods (Table III) is from 18½ days in midsummer to about 25 days in the coolest months. This represents the shortest period elapsing from egg to egg, for oviposition may occur on the day of emergence. This is a slightly shorter average period than obtains in the life of the fruit fly.

TABLE III.—*Duration of the combined egg, larval, and pupal stages of Diachasma tryoni and Opius humilis*

Diachasma tryoni.[a]					*Opius humilis*.				
Date.	Number individuals under observation.	Duration.		Mean temperatures.	Date.	Number individuals under observation.	Duration.		Mean temperatures.
		Extremes.	Average days.				Extremes.	Average days.	
1917				°F.	1916				°F.
Jan.	200	21–29	24.5	70.5	Jan.	34	18.5–23.5	20.5	71.0
Feb.	56	23–30	25	70.2	Feb.	177	17.5–25.5	20	72.6
Mar.	122	21–30	23.5	71.9	Mar.	83	17.5–23.5	19.5	72.2
					Apr.	208	16–21	17.9	74.2
1916					May.	258	15–21	17	75.2
Apr.	56	19.5–24.5	21	74.2	June.	46	13.5–17.5	15.5	75.6
May.	60	18.5–25.5	20	75.2	July.	42	13.5–16.5	14.5	76.8
June.	522	17.5–24.5	20	75.6	Aug.	104	14.5–17.5	15.5	76.6
July.	745	16–24	18.5	76.8	Sept.	133	14.5–17.5	15.5	77.3
Aug.	428	17–23	18.5	76.6	Oct.	100	15–19	16	76.6
Sept.	1,557	17–25	18.5	77.3	Nov.	158	15.5–20.5	17.5	75.0
Oct.	751	17.5–24.5	20	76.0	Dec.	104	18–23	19	72.4
Nov.	711	18.5–27.5	20.6	75.0					
Dec.	493	20–28	22.5	72.4					

[a] This table does not include hibernating individuals.

ADULT

The adult extricates itself from the host puparium by actively gnawing the part directly in contact with the head. In opening and closing the mandibles a transverse cut is made, usually in the third or fourth pupal segment and extending around about one-third of the circum-

ference. In pressing and working the head through this cut the entire end of the puparium usually is broken off and the parasite quickly emerges (fig. 17). In a few moments the antennæ, legs, and ovipositor straighten out. Immediately upon emerging the meconium is discharged. The period in the development of parasitic Hymenoptera when the meconium is discharged is interesting. With the opiines treated in this publication the meconium is never voided until the adult emerges. In the eulophid *Tetrastichus giffardianus* a major meconium is discharged just after the emergence of the adult and there is a barely perceptible discharge during the prepupal period. The pteromalid *Pachycrepoideus dubius* and the proctotrupid *Galesus silvestrii* void a large quantity of waste while in the prepupal stage and later, upon emergence, void an insignificant meconium.

Males nearly always commence emerging before the females and usually are all out while the females are still actively emerging. The period of greatest emergence of the males is from two to three days earlier than is that of the females. After leaving the puparium nothing can be found of the pupal skin. It is so extremely thin as to be almost invisible in water beneath a cover glass. Before the adult emerges, however, the pupal skin can be torn from the pupa as a thin, transparent covering.

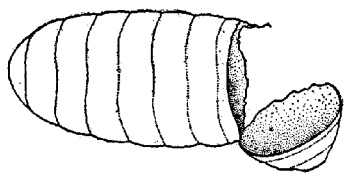

FIG. 17.—*Diachasma tryoni*: Fruit fly puparium showing emergence hole made by adult parasite. Length 4 mm. Typical of exit hole made by *D. fullaway*i and *Opius humilis*.

Copulation takes place most frequently during the first few days after emergence. In the laboratory mating has been repeatedly observed within 5 to 10 minutes after the adults have come out. The copulation period is short. It lasts from about 10 to about 60 seconds. The sex attraction is most strongly evident in the male. Practically all activity prior to and coincident with mating is on the part of the male. It becomes greatly excited when within 1 to 3 inches of the female and vibrates the wings rapidly and spasmodically. The male emits a strong, sweet odor. It is greatest in intensity in the presence of the female. No perceptible odor issues from the female. It never shows any great interest in the male and can readily repel it with the posterior pair of legs. In glass tubes the males make no distinction between mated and virgin females. Weak or injured females, unable to repel the male, may mate an indefinite number of times. A male may successfully copulate with different females more than once within a short period. A freshly emerged male was observed to mate with different females on August 24, 1917, at 10.20 a. m.,[1] 10.25 a. m., and 11.05 a. m. Normal

[1] All references to clock time relate to "standard time."

females confined with males in large sterilizing tubes have been observed in copulation as many as four to seven times during a given hour. Mating is probably best secured with the parasites confined in large screened cages a foot or more in diameter. Large cages with plenty of light are certainly superior to glass tubes for this purpose, although mating will occur in small shell vials. Under certain conditions males of *Diachasma tryoni* have mated with females of *D. fullawayi*. This may be brought about by confining many fresh males of *tryoni* with a few females of both *tryoni* and *fullawayi*. The progeny from two *fullawayi* females mated in this manner on June 29, 1916, were all males. Evidently these two species can not be crossed.

Males are more abundant than are females. During 1916 and 1917, 16,845 males and 10,130 females were bred from fruit-fly material collected in the field. Experiments conducted in the laboratory show that mated females produce a varying proportion of male and female progeny. From 11 mated females emerging on July 6 and 7 and August 24, 1917, and immediately isolated into separate cages for oviposition until death, the following progeny were secured: No. 1, 13 males and 23 females; No. 2, 24 males and 7 females; No. 3, 19 males and 27 females; No. 4, 5 males and 9 females; No. 5, 7 males and 21 females; No. 6, 14 males and no females; No. 7, 4 males and no females; No. 8, 49 males and 10 females; No. 9, 16 males and 15 females; No. 10, 23 males and no females; No. 11, 15 males and 2 females. In another case 25 freshly emerged females were placed separately with males on July 13, 1917, and left until all had mated. From these a total of 189 males and 86 females was reared. Three females, known to have mated with vigorous males four, five, and seven times, respectively, were given opportunity to oviposit from August 9, 1917, until death from three to four weeks later. From these a total of 47 males and 24 females was reared. The female which had mated seven times produced 14 males and 4 females. Such data indicate the importance of other factors than mere successful mating in the determination of sex proportion.

Unmated females of *Diachasma tryoni* as well as the other two opiine species treated herein are positively arrhenotokous. Ovipositing virgins during 1916 selected to prove this point consistently produced nothing but males.

Females may begin oviposition on the day of emergence, irrespective of whether they have mated or not. The ovaries are well filled with eggs, in a mature condition, at the time of emergence. Eggs are also present in varying degrees of development (fig. 19). A dissection of the ovaries of 24 females 24 hours old gave an average of 84 mature eggs per female. The greatest number of mature eggs found in a single day-old female was 125. Females which have been hibernating in the larva stage from 3 to 12 months are fertile, mate and reproduce, but are not

so prolific as are females coming from nonhibernating larvæ. The longer the larvæ have undergone hibernation, the weaker the reproductive system when the female finally emerges. An examination of the ovaries of nine females during 1916 and 1917, originating from larvæ hibernating from 3 to 12 months, gave an average of 37 mature eggs per female. Examination of the ovaries of two females originating as larvæ that had hibernated 12 months disclosed only 9 and 13 eggs, respectively. Examination of the ovaries of three females maturing from larvæ which had lain dormant for 10 months disclosed 31, 10, and 24 mature eggs, respectively.

OVIPOSITION

The maximum number of eggs usually are deposited during the first week of the life of the female. As many as 30 eggs have been deposited by a single female in a given day. A healthy female usually deposits from 5 to 9 eggs daily for the first week and only a few eggs daily thereafter. Death usually follows a few days after the cessation of egg-laying. The largest number of eggs deposited by a female in confinement was 148 (Table IV). This female lived only 12 days and died with 54 mature eggs in the ovaries. As noted later, ovipositing females do not live as long as do individuals given no opportunity to oviposit. Only one egg is deposited at a time. The total operation of laying a single egg requires from 15 to 45 seconds after the ovipositor has penetrated the fruit and located a host larva. A female may deposit an egg, rest a moment, and oviposit into the same or another larva immediately, but the ovipositor is completely removed after the placing of each egg. In ovipositing, the female moves about over the fruit, frequently pausing and moving in a circle over certain spots. The location of the larva, lying invisible in the fruit, appears to be wholly through a sense of touch. Judging from the actions of the female, all movements over the surface of the fruit indicate attempts to detect vibrations on the surface due to movements of the larva beneath the skin. When a larva is located, the female elevates the abdomen to an angle of about 45° and the ovipositor is brought to an almost vertical position with the tip resting against the fruit beneath the body. (Pl. 32, A.) The parasite never enters a broken fruit or penetrates into exposed pulp containing larvæ, all oviposition being entirely from the surface. Upon the insertion of the ovipositor the larva usually attempts to escape and frequently does. Barbs on the end of each of the two sharp, piercing ovipositor blades (fig. 18, *c*, *d*) probably enable the female to hold the larva in position, once the blades are fairly inserted. A third blade (fig. 18, *b*), which enters with the two piercing blades, but which is not sharp or barbed, is specifically designed for conveying the acid or poison from the acid and alkaline glands (fig. 19, *a*, *b*). The head end of this blade bears numerous perforations which are the surface openings of minute branching channels leading from the large central, hollow part of

this blade, through which the toxin flows during oviposition. A slight temporary paralysis of the larva seems to result after the ovipositor is fairly inserted, for little struggling ensues after the egg is extruded. The two other parts of the ovipositor, in addition to the piercing blades, consist of the outer pair of hollow sheaths (fig. 18, *a*, *e*), which do not penetrate beyond the surface of the fruit during oviposition unless the larva attacked lies under a large break or hole in the fruit. Several hours after

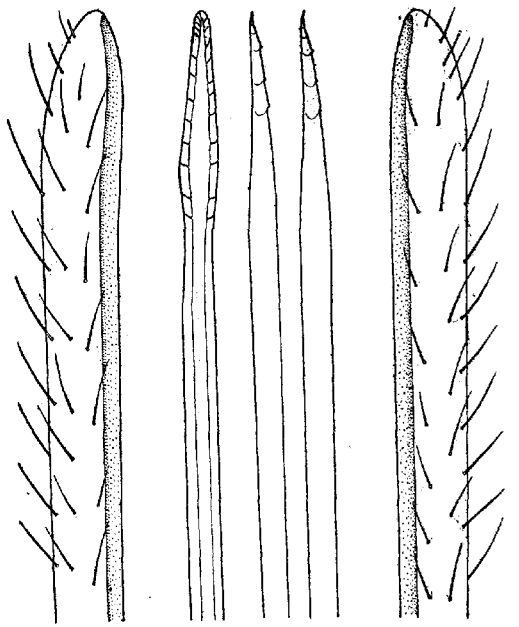

FIG. 18.—*Diachasma tryoni*: Parts of ovipositor: A, E, lateral sheaths; B, poison blade; C and D, piercing blades, showing characters of ends of each blade. Greatly enlarged.

a larva has been stung, a minute, oval, brownish spot develops on its body at the point where the ovipositor was inserted. This spot becomes a permanent scar and can be distinctly seen on the surface of the puparium. The female exhibits no decided capacity for discerning parasitized from unparasitized larvæ. Superparasitism is thus very common, although only one parasite ever develops in a superparasitized larva. This is owing to the cannibalistic habits of newly hatched larvæ of *Diachasma tryoni*.

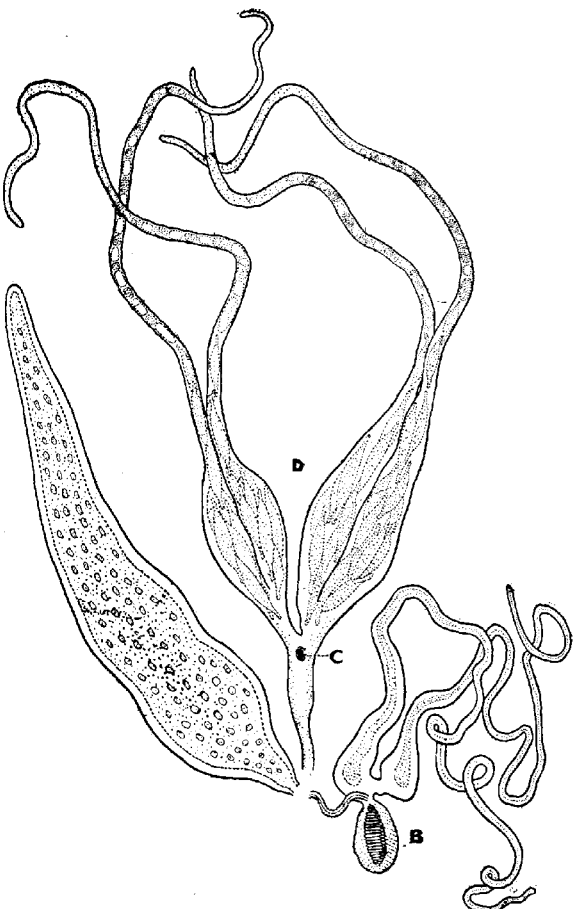

FIG. 19.—*Diachasma tryoni:* Reproductive system of newly emerged female: A, alkaline gland; B, poison reservoir with poison glands leading to it; C, spermatheca; D, ovaries, showing position and usual number of eggs and developing egg cells in newly emerged female. Greatly enlarged.

TABLE IV.—*Daily rate of oviposition of Diachasma tryoni, 1917*

Date of oviposition.	Number of eggs deposited.								
	No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.	No. 7.	No. 8.	No. 9.
May 18	.....	16	26	24	19	.....	.....	.....	.....
19	21	.....	11	12	3	.....	.....	.....	.....
20	22	6	17	5	15	.....	.....	.....	.....
21	8	16	.....	23	18	.....	.....	.....	.....
22	27	1	7	6	20	.....	.....	.....	.....
23	23	1	.....	.....	7	.....	.....	.....	.....
24	7	7	.....	7	30	.....	.....	.....	.....
25	1	4	.....	.....	14	.....	.....	.....	.....
26	.....	.....	.....	1	4	.....	.....	.....	.....
27	.....	.....	.....	Died	18	.....	.....	.....	.....
28	Died	Died	.....	.....	Died	.....	.....	.....	.....
June 5	.....	.....	Died	.....	.....	.....	.....	.....	.....
July 19	.....	.....	.....	.....	.....	3	.....	.....	.....
20	.....	.....	.....	.....	.....	5	2	.....	.....
21	.....	.....	.....	.....	.....	10	17	.....	.....
22	.....	.....	.....	.....	.....	8	13	.....	.....
23	.....	.....	.....	.....	.....	9	9	.....	.....
24	.....	.....	.....	.....	.....	13	21	.....	.....
25	.....	.....	.....	.....	.....	14	5	.....	.....
26	.....	.....	.....	.....	.....	10	.....	.....	.....
27	.....	.....	.....	.....	.....	9	5	.....	.....
28	.....	.....	.....	.....	.....	1	1	.....	.....
29	.....	.....	.....	.....	.....	4	.....	.....	.....
30	.....	.....	.....	.....	.....	10	1	.....	.....
31	.....	.....	.....	.....	.....	4	1	.....	.....
Aug. 2	.....	.....	.....	.....	.....	.....	Died	.....	.....
Oct. 9	.....	.....	.....	.....	.....	Died	.....	.....	.....
10	.....	.....	.....	.....	.....	.....	.....	2	.....
11	.....	.....	.....	.....	.....	.....	.....	6	12
12	.....	.....	.....	.....	.....	.....	.....	1	6
13	.....	.....	.....	.....	.....	.....	.....	15	15
14	.....	.....	.....	.....	.....	.....	.....	13	10
15	.....	.....	.....	.....	.....	.....	.....	12	8
16	.....	.....	.....	.....	.....	.....	.....	4	7
17	.....	.....	.....	.....	.....	.....	.....	7	7
18	.....	.....	.....	.....	.....	.....	.....	5	5
19	.....	.....	.....	.....	.....	.....	.....	7	4
20	.....	.....	.....	.....	.....	.....	.....	2	1
23	.....	.....	.....	.....	.....	.....	.....	.....	7
25	.....	.....	.....	.....	.....	.....	.....	.....	12
26	.....	.....	.....	.....	.....	.....	.....	Died	.....
27	.....	.....	.....	.....	.....	.....	.....	.....	2
28	.....	.....	.....	.....	.....	.....	.....	.....	6
30	.....	.....	.....	.....	.....	.....	.....	.....	1
31	.....	.....	.....	.....	.....	.....	.....	.....	1
Nov. 8	.....	.....	.....	.....	.....	.....	.....	.....	Died
Total	109	51	61	78	148	100	75	74	104

No. 1 to 5, inclusive, emerged on May 13 and were given fruit-fly larvæ in which to oviposit on May 18 and daily thereafter.

Nos. 6 and 7 emerged July 18 and Nos. 8 and 9 emerged on October 10.

No. 1 died with 10 mature eggs in the ovaries.
No. 2 died with 22 mature eggs in the ovaries.
No. 3 died with 0 mature eggs in the ovaries.
No. 4 died with 10 mature eggs in the ovaries.
No. 5 died with 54 mature eggs in the ovaries.
No. 6 died with 5 mature eggs in the ovaries.
No. 7 died with 38 mature eggs in the ovaries.
No. 8 died with 0 mature eggs in the ovaries.
No. 9 died with 42 mature eggs in the ovaries.

Although the mature fruit-fly larva is the stage most frequently attacked by the parasite, younger larvæ are often sucessfully parasitized. On September 25, 1916, a microscopical examination of the contents of 107 fruit-fly larvæ in the second instar, removed from fruit collected in Honolulu, gave interesting results. Twenty-four of these contained eggs or larvæ of either *Diachasma tryoni* or *Opius humilis*.

The female of *Diachasma tryoni* oviposits in larvæ in fruit after it has fallen to the ground and with equal facility attacks the larvæ in the fruit before it falls. The heaviest parasitism, however, probably occurs while the fruit is still on the tree. In March and April, 1917, a quantity of fruit; infested with fruit-fly larvæ known to be unparasitized, was placed on the ground beneath fruit trees and left for periods of 24 to 48 hours. It was then collected and placed in glass jars. From this fruit 208 individuals of *D. tryoni* were reared. A total parasitism by *D. tryoni* of 27.1 per cent was secured during August, September, and October, 1917, from 1,435 fruit-fly puparia coming from fruit collected from the trees in Honolulu. From 15,907 fruit-fly puparia secured from fruit collected from the ground during the same months a parasitism of 31.1 per cent by *D. tryoni* was obtained.

Of 83,304 fruit-fly larvæ secured in Hawaii during 1916, 13.3 per cent were parasitized by *Diachasma tryoni*. During 1917, as determined from collections of 72,139 larvæ, the parasitism by this species was 20.3 per cent.

Parasites confined in glass sterilizing tubes closed at one end, plugged with cotton, and kept continuously in partial darkness usually will remain alive and active for about two months. After eight or ten weeks of confinement the mortality is heavy. Only a few individuals have been kept alive beyond 80 days. Females not permitted to oviposit generally outlive the males. There is one record, however, of a male that lived for 125 days. Ovipositing females do not live in confinement much beyond 25 days even under the best of care. During May and June, 1917, 98 females were confined in small screened boxes kept in partial darkness and given material in which to oviposit constantly. Of these, 87 lived from 12 to 18 days and only 11 lived from 20 to 24 days. These were fed daily with a thin solution of honey and water placed in minute drops upon fresh leaves. Brown sugar, diluted with water in a ratio of 1 part of sugar to 5 of water, is a satisfactory food, though the results of feeding with diluted honey are better. A small portion of crushed apple or other fruit is relished by the parasites. Extract of beef added to the honey solution has been tried with unsatisfactory results. Concentrated honey or sugar solutions are also unsatisfactory. Parasites have been kept in a vigorous state longest when given honey diluted with 4 or 5 parts of water varied with a 3- or 4-hour period daily during which nothing but water is given.[1] Without food and held in bright light, the majority of the adults of this species under observation died in from 50 to 60 hours after emergence.

[1] For useful methods of confining parasites see Pemberton and Willard (4).

OPIUS HUMILIS

The parasite *Opius humilis* was brought to Honolulu from West Africa by Silvestri in May, 1913. It was soon established in the Kona coffee district of the Island of Hawaii, owing to the liberation there of a few individuals in June, 1913. By October of the following year it was found frequently parasitizing from 80 to 95 per cent of the larvæ developing in coffee in this district. Its general distribution and value were proven in Honolulu several months previously. As shown by data published elsewhere by the writers, the importance and effectiveness of this parasite soon became greatly curtailed through the restraint operated over it by the other introduced parasites.

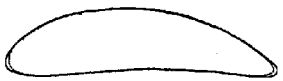

FIG. 20.—*Opius humilis*: Egg freshly laid. Length 0.48 mm.

DESCRIPTION AND LIFE HISTORY

EGG

When first deposited (fig. 20), the egg is cylindrical, transparent, with smooth glistening surface, slightly curved and bluntly pointed at each end. The cephalic end is less pointed than is the opposite end. It is 0.48 mm. long and is nearly one-fifth as wide as long. No tubercular protuberances are present at either end when first laid nor is the outer enveloping membrane present that surrounds the egg of *Diachasma tryoni*. When fully developed it is 0.85 mm. long and less than one-third as broad as long (fig. 21), and is hardly recognizable as the egg deposited two days previously. Each end is prolonged into a distinct tubercle, the caudal end being prolonged much more than the opposite end. As in *D. tryoni*, the egg is placed just beneath the surface of the larval skin but so far as to be invisible from the surface. The wound made on the larva by the ovipositor remains permanently as an oval, brown scar.

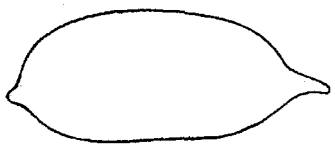

FIG. 21.—*Opius humilis*: Mature egg. Length 0.85 mm.

The duration of the egg stage ranges from about 45 hours in the summer months to 53 hours in the winter months. (For temperatures see Table III.) This is an average of about nine hours shorter than the similar period for *Diachasma tryoni*. Ten eggs deposited on March 24, 1917, at 10 a. m. hatched 48 hours later. One hundred and sixty-two eggs deposited on July 6, 1917, between 12 m. and 2 p. m. hatched from 45 to 47 hours later. Forty-two eggs deposited on May 13, 1916, between 10 a. m. and 12 m. hatched 48 hours later. The differences in duration are due to variations in temperature. The hatching of the egg and the effect of the egg upon the development of the host are identical with that of *D. tryoni*.

LARVA

The newly hatched larva (fig. 22, 23, 24) is almost exactly the same in size, structure, and habit as is the newly emerged larva of *Diachasma tryoni*, with the following exceptions: (1) The two pointed teeth situated at the middle of the cephalic edge of the chitinized ventral plate of the head are closer together than in *tryoni* and are joined basally to form a smoothly rounded letter "U"; (2) in *tryoni* the "U" formed by these two teeth is somewhat squarely made (compare fig. 3 and 24); (3) the head is somewhat smaller and squarer than is that of *tryoni;* (4) the ventral mass of serosal cells, retained after the hatching of the larva, is much smaller in volume, less conspicuous, and is often broken away from the larva before the latter molts to the second instar. The duration of this instar and the circumstances influencing the duration are almost identical with those of *D. tryoni.*

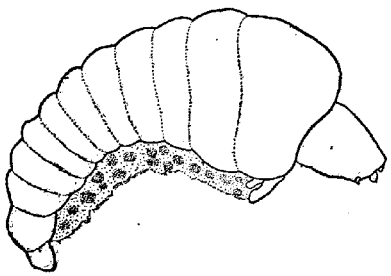

FIG. 22.—*Opius humilis:* Larva of first instar, lateral aspect, showing position and quantity of egg serosal cells clinging to ventral surface. Length 1.2 mm.

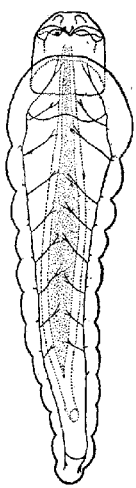

FIG. 23.—*Opius humilis:* Larva of first instar, dorsal aspect, showing head characters, complete tracheal system, and digestive canal. Length 1.1 mm.

The characters, habits, internal development, and duration of the second and third larval instars are so similar to those of *Diachasma tryoni* as to need no special comment. With the molt to the fourth instar, however, some distinguishing characters are readily seen. The mandibles are smaller than are those of *D. tryoni*, being 0.065 mm. long, more narrowly pointed, and wholly lacking in chitinization at the base (fig. 25). The dark chitinized ring at the base of the mandibles of the mature larva of *D. tryoni* immediately distinguishes it from larva of *Opius humilis* in the same instar. The period of the mature larva is short and does not extend much beyond five days. The larva never hibernates. As a large number of larvæ of species of Diachasma hibernate throughout the year, the absence of this trait in *O. humilis* renders it a more prolific parasite, in conjunction with its other characters, than is either of the two species of Diachasma. There are four larval instars.

PUPA

The pupa may be distinguished from the pupa of *Diachasma tryoni* by the short antennæ and ovipositor sheath. The ovipositor sheath extends beneath the body and up over the abdomen but the tips do not reach as far forward as the thorax. The pupa stage is slightly shorter than is that of *D. tryoni*.

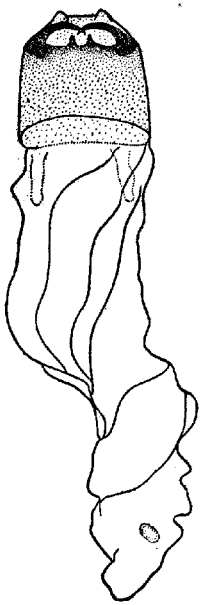

FIG. 24.—*Opius humilis:* Molted skin of first-instar larva, showing head characters. Length 1 mm.

Although the egg, larva, and pupa stages are slightly shorter than are those of *D. tryoni*, the average period covering their combined development is consistently shorter by 3t o 4 days (Table III). During the summer months this combined period averages 15½ days and increases to about 20½ days in the winter. As eggs are deposited by the female on the day of emergence, the life cycle of this species covers a distinctly shorter average period than does that of its host.

ADULT

The emergence of the adult is similar to that of *Diachasma tryoni*. Males likewise precede the females by a day or more. The meconium is immediately discharged, as in *D. tryoni*. The pupal skin is extremely thin and difficult to see. The general habits of mating are identical with those of *D. tryoni*. The male emits a distinct but rather delicate sweet odor. No odor can be detected on the female. The proportion of sexes is better equalized than is the case with *D. tryoni*. During 1916 and 1917 a total of 6,128 males and 4,715 females was reared from material collected in the field. This is a percentage of 56.5 of males as compared with a percentage of 62.4 of males of *D. tryoni* secured over the same period.

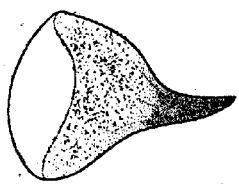

FIG. 25.—*Opius humilis:* Mandible of mature larva. Length 0.065 mm.

In confinement no conditions could be obtained under which this species would reproduce as favorable a proportion of the sexes as occurs in the field. The best results were obtained with females confined in a large glass jar (9 by 15 inches). In one experiment 25 males and 25 females were placed in such a jar immediately upon emergence, kept in strong light, and daily given material in which to oviposit. In three days' time mating and oviposition oc-

curred, resulting in the rearing of 114 males and 52 females. It has been found possible to rear reasonable quantities of females under various conditions of confinement. A large, well-lighted glass or screen cage is strongly recommended. Cages less than 9 or 10 inches in diameter, or even small glass tubes, can always be safely used for ovipositing females, but satisfactory mating does not occur in small cages. From 100 males and 75 females placed, upon emergence, in a small cage having a diameter of 2½ inches and a length of 7 inches, a total of 558 males and 27 females was reared during their lifetime. This is an average result in rearing this species when the cage is small. It shows an abundant oviposition but little mating.

Unmated females reproduce parthenogenetically, the progeny being always males. These males have been proved fertile.

OVIPOSITION.

Oviposition may commence upon the day of emergence. An average of 80 mature eggs occurs in the ovaries of the newly emerged female. The manner of egg deposition is similar to that described for *Diachasma tryoni*. Only one egg is deposited at a time. An average period of 17 seconds is required for the deposition of a single egg, after the ovipositor has penetrated the fruit and located a larva. This is based upon the timing of 31 separate egg depositions. The shortest time was 10 seconds and the longest 3 minutes and 15 seconds. The female attacks larvæ in fruit on the ground as well as larvæ in fruit on the tree, she may oviposit into fruit-fly larvæ in the second instar, and selects no particular part of a larva in which to oviposit. The daily rate of oviposition is indicated in Table V. Female 6 therein is of unusual interest. A total of 255 eggs, deposited quite generally over a period of 20 days, indicates that this species oviposits probably over a longer period than does *D. tryoni*. The greatest number of eggs deposited in 24 hours was 34. It is of interest that most of the individuals shown in Table V died shortly after the last egg was deposited. Female 6 was given, in addition to honey and water in the proportions of 1 part honey to 6 of water, a daily feeding of pure water. During the morning hours nothing but water was given, the honey being added in the afternoon. All of the parasites used in the oviposition records were confined in glass cylinders 6 inches long and 1 inch in diameter, open at both ends but protected with screened caps. Unparasitized fruit-fly larvæ reared in the laboratory were used in obtaining the records. The larvæ were daily placed in the fruits of *Mimusops elengi* (Plate 32, A) and were removed daily thereafter and dissected, under magnification, for eggs of the parasite and replaced daily by others so that the experiment might be continued until the death of the females.

TABLE V.—*Daily rate of oviposition of Opius humilis. 1916–17*

Date of oviposition.		Number of eggs deposited.					
		No. 1.	No. 2.	No. 3.	No. 4.	No. 5.	No. 6.
Oct.	10	6					
	11	3					
	12	17					
	13	11					
	14	18					
	15	23					
	16	10					
	17	8					
	18	5					
	20	1					
	25	1					
Nov.	1	Died					
July	6		2	2	3	3	
	7		14	10	6	5	
	8		2	7	2		
	9		32	1	10		
	10		7	1	5		
	11		5	1		4	
	12		15	14	19	10	
	13		19	8	1	8	
	14			4	5	1	
	15					1	
	16		28		11		
	17		9	4			
	18			11			
	19		4	5	5		
	20		4	18	12		
	21			6	12		
	22				5		
	23			3			
	24		1	5	3		
	25			6			
	26			4	1		
	27						18
	28						24
	29						24
	30			Died.	Died.		10
	31						25
Aug.	1						34
	2						22
	3						16
	4						10
	5					Died.	2
	6						13
	7		Died.				8
	8						6
	9						6
	10						8
	11						5
	12						4
	13						11
	15						9
	23						Died.
	Total	109	142	110	100	32	255

No. 1 emerged on October 10, 1916.
Nos. 2, 3, 4, and 5 emerged on July 6, 1917.
No. 6 emerged on July 27, 1917.
No. 1 died with 14 mature eggs in the ovaries.
No. 2 died with 8 mature eggs in the ovaries.
No. 4 died with 18 mature eggs in the ovaries.
No. 6 died with 10 mature eggs in the ovaries.
No examination was made of the ovaries of Nos. 3 and 5 at death.

Superparasitism in the field is common and indiscriminate, no preference for parasitized or unparasitized larvæ being evident.

The ovipositor blades of this species, though hardly one-third as long as those of *Diachasma tryoni*, are otherwise almost identical in structure, position, and number.

Of 83,304 fruit-fly larvæ secured in Hawaii during 1916, 17.2 per cent were parasitized by *Opius humilis* and during 1917, as determined from collections of 72,139 larvæ, parasitism by this species was 12.7 per cent.

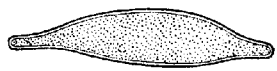

FIG. 26.—*Diachasma fullawayi:* Freshly deposited egg. Length 0.33 mm.

Individuals held in glass sterilizing tubes and kept in partial darkness lived somewhat longer than did adults of *D. tryoni*, when fed and held under the same conditions. Of 541 individuals confined with diluted honey and water as food, 18 females lived 100 days or more, two of these living until 125 days old.

DIACHASMA FULLAWAYI

Diachasma fullawayi was brought to Honolulu by D. T. Fullaway in October, 1914. The material was secured in West Africa by D. T. Fullaway and J. C. Bridwell. It was originally found in West Africa by Silvestri in 1912.

DESCRIPTION AND LIFE HISTORY

EGG

When first deposited, the egg (fig. 26) is about 0.33 mm. long and 0.066 mm. wide. At maturity (fig. 27) it is about 0.66 mm. long and 0.22 mm. wide. Other than being slightly broader than is the egg of *D. tryoni*, it is almost identical with the latter in development, shape, color, size, duration of stage, and manner of hatching.

FIG. 27.—*Diachasma fullawayi:* Mature egg. Length 0.66 mm.

LARVA

The newly hatched larva has been found to differ from that of *D. tryoni* in one noticeable respect. The cephalic edge of the chitinized ventral portion of the head bears three pointed teeth instead of two, as in *D. tryoni*. The middle tooth is less than one-half as long as the other two (fig. 28). The color of the chitin in the head is a shade darker than in *D. tryoni*. Otherwise the two larvæ are practically identical in appearance, movements, internal structure, feeding habits, and duration of the instar.

The larvæ of the second and third instars have not been distinguished in any respect from those of *D. tryoni*. The mature larva, which is the fourth instar, differs from the *tryoni* larva only in being faintly yellowish in color and in having a slightly darker chitinization of the mandibles (fig. 29). The mature larva of *D. fullawayi* also hibernates, but the percentage of larvæ hibernating appears to be less than occurs with *D. tryoni;* the period of hibernation also is shorter. The greatest amount of adult emergence from hibernating individuals occurs during the first three months after the larva matures. From 68 larvæ passing into a state of dormancy between August 1, 1916, and July 31, 1917, inclusive, 29, 16, 21, and 1 pupated and became adults during the period from the first to the fourth months, respectively. One individual hibernated for 8 months and 15 days. The greater proportion of the larvæ under observation went into hibernation during the months of November, December, January, February, and March, and the greatest period of adult emergence from hibernating material was in March, April, and May. A much greater degree of hibernation occurs in fruit-fly puparia left in soil or sand than obtains in dry glass vials.

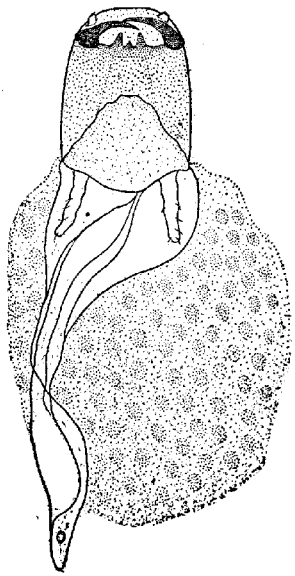

FIG. 28.—*Diachasma fullawayi:* Cast skin of first-instar larva, showing head characters and egg serosal cells still clinging to ventral surface. Length 1 mm.

PUPA.

The pupa may be distinguished from that of *D. tryoni* by the unusually long ovipositor sheath which extends back over the body almost to the head. The duration of the pupa stage is the same as that of *D. tryoni*, as is also the duration of the combined egg, larva, and pupa stages.

ADULT

The behavior of *D. fullawayi* after general distribution in Hawaii is interesting. Localities having high humidity and precipitation have proven especially favorable for this species. At points where the average humidity is low it has had evident difficulty in existing at all, even under very favorable host conditions. An investigation of this species in 1917, after wide distribution and thorough establishment, clearly indicated the particular capacity of this species for life under

humid conditions. Of 2,232 fruit-fly puparia collected in the Kona coffee district of the island of Hawaii in 1917, 2 per cent were parasitized by *D. fullawayi* and the percentage of parasitism here, where the precipitation averages about 50 inches a year, was about the same in 1916. On the opposite side of the island, about the town of Hilo, where the precipitation averaged 200 inches during 1916, parasitism by this species reached 60 per cent, as determined from 316 fruit-fly puparia collected from coffee. During 1917 an unprecedented drought was experienced on the Hilo side of the island and parasitism by *D. fullawayi* was reduced to a fraction of 1 per cent. From 700 fruit-fly puparia secured from that source during November, 1917, at the time of the drought, no individuals of *D. fullawayi* were reared, although the other opiines were abundant, particularly *Opius humilis*. Fifteen miles from Hilo, however, in a locality more elevated and forested, where the humidity can not get very low, the parasitism by *D. fullawayi* was 98.2 per cent, as determined from a collection of 259 fruit-fly puparia secured from coffee on the same date as that on which the Hilo collection was made. Perhaps the most significant evidence that can be given to bear out this point is the parasitism by this species during 1917 in upper Manoa Valley in Honolulu and at the Maunawili Ranch, Oahu. At two localities the precipitation averages 150 inches a year and at Maunawili the Weather Bureau records show an average of 324 rainy days a year. From 78 fruit-fly puparia collected at Maunawili during 1916 the parasitism by *D. fullawayi* was 65.3 per cent. From 1,542 puparia secured at that place in 1917 the parasitism by this species was 88.4 per cent. From 474 puparia secured from coffee in upper Manoa Valley in 1917 the parasitism by *D. fullawayi* was 91 per cent. Honolulu, on Oahu, has a precipitation closely paralleling that of the Kona district on the island of Hawaii, usually averaging 50 inches a year. In Honolulu the average collections of fruit produce fruit-fly larvæ only slightly parasitized by *D. fullawayi*, even though upper Manoa Valley is only a few miles removed from Honolulu. Still the difference in rainfall is great between the two localities.

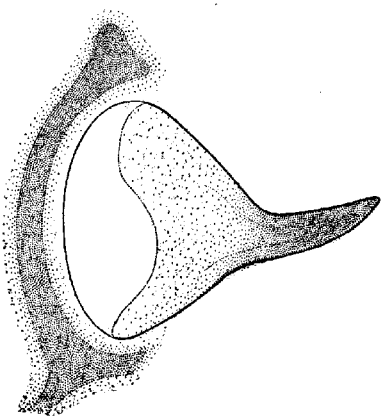

FIG. 29.—*Diachasma fullawayi*: Mandible of mature larva. Length 0.12 mm.

In addition to being especially adapted for propagation in wet areas, this parasite, for some unexplained reason, favors certain fruits harboring the host larvæ. It is produced in Honolulu most frequently from fly puparia secured from the loquat (*Eriobotrya japonica*), bestill (*Thevetia neriifolia*), French cherry (*Eugenia uniflora*), coffee, and the fruits of *Chrysophyllum monopyrenum*. It will frequently attack larvæ in these fruits to the almost total exclusion of heavily infested fruits of the kamani that may be growing close by. It particularly favors the loquat.

Emergence, mating, and oviposition are not different, so far as can be noted, from these habits as described for *D. tryoni*. No odor is emitted by the male or female. A meconium is discharged immediately after emergence. The females may reproduce parthenogenetically, the progeny being always males.

Adults of this species have been kept alive longer than have any of the other parasites. Between December, 1916, and May, 1917, of 235 adults held in confinement in glass tubes and fed honey and water 43 lived over 100 days, 11 lived 120 days or over, 1 lived for 134 days, and 1 for 141 days. Most of the long-lived individuals were females. Three males lived for 120, 121, and 127 days, respectively. Without food and held in bright light the majority of the adults die in about 55 hours. One male has been kept alive for 103 hours and 2 females for 79 hours.

The proportion of sexes is more favorable in this species than in the case of either *D. tryoni* or *Opius humilis*. During 1916 and 1917 a total of 5,528 males and 5,566 females emerged from fruit-fly material collected in the field.

Of 83,304 fruit-fly larvæ secured in Hawaii during 1916, 2.1 per cent were parasitized by *D. fullawayi*. During 1917, as determined from collections of 72,139 larvæ, the parasitism by this species was 7.3 per cent.

TETRASTICHUS GIFFARDIANUS

This species was brought to Honolulu from West Africa by Fullaway in October, 1914. It was collected and colonized in Africa by Fullaway and Bridwell. It soon became largely propagated and widely distributed in the Territory of Hawaii. Though its life cycle is short and its development as rapid as is that of the opiines, its importance as a parasite prior to 1917 was doubtful. During 1917, however, it had become better established and had increased very considerably the total parasitism of the fruit fly about Honolulu in fruits having thick pulps, such as oranges, peaches, and guavas. Larvæ in these fruits are not easily reached by braconid parasites. It is the most prolific of any of the introduced parasites of the fruit fly, and, under favorable host and weather conditions, may multiply enormously in localized spots. Owing to the short life of the adult, the absence of a hibernating form, its small size, and seemingly poor powers of flight, it rapidly drops off in effectiveness as soon as host material becomes scarce within a short radius.

DESCRIPTION AND LIFE HISTORY

EGG

The newly deposited egg measures from 0.20 to 0.25 mm. in length, is less than a third as broad as long, is pale white, smooth, glistening, cylindrical, and broadly rounded at each end (fig. 30). The eggs are placed in clusters just beneath the larval integument. They are faintly visible from the surface as dark bodies if strong light is transmitted through the larva from below. The egg does not undergo much change in size as the development of the embryo progresses. The duration of the egg stage is from 2 to 2½ days. Of 400 eggs deposited January 4, 1917, at 11 a. m., all hatched in about 60 hours. Fruit-fly larvæ containing eggs of this parasite develop to maturity and form a perfect puparium, in the same manner as do the other parasites under discussion. No fly pupa is formed after the histolysis of the larval tissues in the puparium, and the egg may hatch either before or after the puparium is formed.

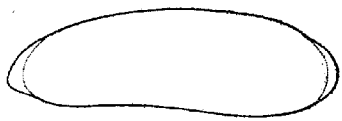

FIG. 30.—*Tetrastichus giffardianus:* Egg newly deposited. Length 0.25 mm.

LARVA

There is nothing of unusual interest in the development of the larva, and only little external difference in the appearance of the instars, with the exception of the gradual increase in size. When first hatched, the larva is about 0.25 mm. long (fig. 31). The body is composed of 13 segments and head, the latter inconspicuous and not unlike the other body segments in general appearance. The mandibles are minute, short, curved, broad at the base, and well chitinized. With only slight modification they are constant in the succeeding instars. Most of the food ingested by this parasitic larva consists of fat bodies. The larva bears 9 pairs of small open spiracles on body segments 2 to 10, inclusive. The tracheal system is open and becomes air filled as soon as the larva hatches. In the first instar the trunks and branches are very faint and threadlike. The larva can move only very slowly and sluggishly in all of its instars.

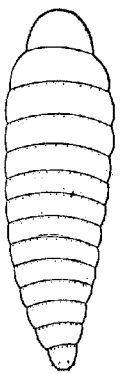

FIG. 31.—*Tetrastichus giffardianus:* Newly hatched larva. Length 0.25 mm.

The number of larval instars has not been determined, though three forms have been distinguished. The mature larva may be distinguished readily by the large, heavy tracheæ and stigmata. As 8 or 10 larvæ nearly always develop in a single host individual, and the food is rapidly consumed, the necessity for well-developed tracheæ is thus apparent

even within a few days after the larva hatches. No cannibalism occurs among the larvæ of this parasite although from 20 to 30 individuals may be developing simultaneously. In such congested cases they nearly all mature, but result in dwarfed adults. No waste matter is passed by the larva, the midintestine being closed caudally during the entire period of larval development. The larva enters a short prepupal period, from 2 to 3 days in duration, before pupating. During this prepupal period a very small portion of the accumulated waste is voided. This would indicate that the mesenteron and proctodeum become connected even before the pupa is actually formed. No hibernating form of the larva is known to occur.

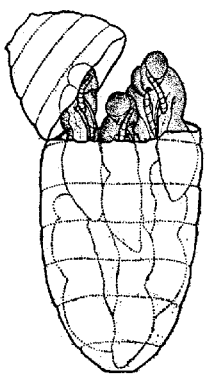

FIG. 32.—*Tetrastichus giffardianus:* Pupæ in normal position and number in fruit-fly puparium opened to show contents. Greatly enlarged.

The pupa is formed with its head facing the cephalic end of the puparium (fig. 32). In cases where 20 or 30 pupæ are packed within the puparium, a few may lie with the position reversed. Of 200 puparia containing pupæ of *T. giffardianus*, examined during August and September, 1917, 3 per cent of the pupæ were lying with the head in this reversed position.

The duration of the combined egg, larva, and pupa stages is from 24 to 31 days in the cool months and lasts about 18 days during the warm months. In January, 1917, 243 adults emerged in from 24 to 31 days after the eggs were deposited, with an average period of 28 days. In April, 1917, 894 adults emerged in from 19 to 26 days after the eggs were deposited, with an average period of 21 days, and in July, 1917, 455 adults emerged in from 17 to 19 days after the eggs were deposited.

ADULT

The adult emerges from the puparium by gnawing a small hole, more or less ragged and circular. Though the puparium may contain from 3 to 30 parasites, usually only one emergence hole is made. The typical position of the emergence opening is shown in figure 33. Occasionally two holes are made, and rarely three. They may be at either end of the puparium or between the extremities. More than one emergence hole usually results from the development of an excessive number of individuals in the puparium. In one rare case wherein 39 parasites emerged from a single puparium, three emergence holes were made. A distinct, thin, brownish pupal skin is left in the puparium after the adult has issued. Although wrinkled and twisted, the skin is an exact replica of the mature pupa. The parasites may twist and turn about

in the puparium for many hours before an opening is cut through which they can escape. Males and females all appear to emerge at the same time, for as soon as a sufficiently large exit hole is made, the adult flies come out as quickly as possible, irrespective of sex. The males remain hovering about the puparia but the females immediately crawl away. The meconium, which is developed and retained in the mid-intestine by the larva and held there in the pupa stage, is voided by the adult immediately upon emergence.

Mating occurs as soon as the adults are out of the puparium. The entire process occupies only a few seconds. Females once mated have no difficulty in warding off the males. No mating occurs within the puparium even though the parasites may be actively moving about in it for several hours before they escape. This is proved from the fact that all females taken immediately after emergence and isolated produce parthenogenetic males. One male may mate with several females within a few hours. On September 8, 1916, one male was placed with 8 virgin females and left for four days. Each female was then placed in a separate vial and given opportunity to oviposit. All produced both male and female progeny. That the male is particularly capacitated for frequent mating is to be expected when the proportion of the sexes is considered, the females always greatly outnumbering the males. During 1916 and 1917 a total of 13,114 males and 47,804 females, or 78.5 per cent, emerged from fruit-fly puparia collected in the field about Honolulu.

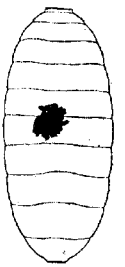

FIG. 33.—*Tetrastichus giffardianus:* Fruit-fly puparium showing characteristic emergence hole made by adult parasite.

Unmated females are always arrhenotokous. From the proportion of sexes secured from field collections, it is shown that the smaller proportion of males readily gain access to and mate with the much larger proportion of females.

OVIPOSITION

Females may begin ovipositing as soon as they emerge, whether mated or not. The mature pupa has well-developed eggs in the ovaries. Fourteen newly emerged females when examined October 20, 1917, contained 73, 70, 72, 60, 73, 66, 71, 81, 65, 45, 58, 64, 61, and 52 mature eggs, respectively. The method of oviposition is best understood by an examination of Plate 32, B, C, and D. The female enters the fruit wherever access can be gained through holes, decayed spots, or breaks on the surface. There is no evidence to indicate that the parasite bores into firm pulp or into the skin of the fruit. Once into the fruit, however, the female may become attached to a larva and be drawn through all manner of pulp and juice before her object has been attained. As soon as a larva is located, the ovipositor is quickly brought forward and beneath the

body and inserted into the body of the larva. In this operation the body is reared up with the front pair of legs stretched at full length or often drawn free from the larva. Only one or two seconds are required for the insertion of the ovipositor. The barbs at the tips of the piercing blades now hold the female securely to the larva. By the time the ovipositor is well inserted the female is standing straight up on the ovipositor and balancing, often on only one posterior leg and the ovipositor or on the ovipositor alone, as shown in the photomicrographs (Pl. 32), after which the female moves very little. The legs and wings are held perfectly still, although the larva may frantically bore about in the pulp or escape entirely from the fruit, in an endeavor to dislodge the parasite. To all contortions the female parasite remains indifferent. When the larva is held in position beneath a microscope a rapid transference of liquid and eggs can be seen passing down the ovipositor. After the eggs are inserted the ovipositor is withdrawn and sheathed in from 2 to 3 seconds. The ovipositor is not removed until the female brings all feet down again in touch with the larva or anything that may be conveniently in reach. The female deposits an average of 8 eggs in a cluster, with each insertion of the ovipositor. Between September 4 and 21, 1916, 50 separate ovipositions were under careful observation. The time elapsing for each oviposition ranged from 9 to 60 seconds, with an average of 35.6 seconds. The number of eggs deposited at each oviposition ranged from 2 to 16 with an average of 8.6. This average is interesting as a laboratory experiment, for it was found that from 3,527 adults of *T. giffardianus* emerging from 412 puparia collected in the field during 1916 and 1917, the average number emerging from these puparia was 8.6 per puparium. It indicates that the parasite generally oviposits only once into the same larva and that the larva is not again stung by another individual, in the majority of cases. One curious point in the rearing of this species from field material should be emphasized. In every case under actual observation wherein a fly puparium produced several females of *T. giffardianus*, one male, and occasionally more than one, developed in the same puparium with them. In other words, each time a mated female places a cluster of 6 to 8 eggs or more in a larva, at least one of those eggs is destined to produce a male, for usually 1 male and 6 or 7 females ultimately emerge.

It has been assumed generally that this parasite works mostly about the ground. Certain evidence has been secured to show that it works a great deal above ground also. Of 94 fruit-fly larvæ emerging from bestill fruits (*Thevetia neriifolia*) picked from trees on August 28, 1917, 18.1 per cent were parasitized by *T. giffardianus*. Of 192 larvæ secured from the same host picked from the trees on August 16, 1917, 9.9 per cent were parasitized by *T. giffardianus*. Forty-five larvæ from the same kind of fruit picked from the trees on September 4, 1917, produced 13.3 per cent *T. giffardianus*. Of 533 fruits of the kamani picked from trees

on October 8, 1917, 7.3 per cent were parasitized by *T. giffardianus*. Parasitism by *T. giffardianus* of larvæ from these fruits, and other varieties picked from the ground on the same dates, was not appreciably higher than that just cited. The individuals reared from larvæ coming from kamani fruits picked from the trees indicate that the females may fly well up above the ground, for the lowest fruits of the kamani are usually from 10 to 12 feet above the ground. It is a high, wide-spreading tree.

Although the female oviposits into any portion of the host larva within reach, the majority of the eggs are placed in the posterior half of the body. This is because the larva buries its head into pulp or juice almost as soon as a female touches it, and, in her attempts to cling to the larva and insert the ovipositor, the attachment is not ordinarily made until she has been drawn back toward the posterior end. The female may oviposit into a larva without being actually in contact with it. This occurs when the ovipositor is forced through thin layers of pulp under which the female may detect the presence of a larva. The larva must be close to the female, however. A distinct, brownish, oval scar remains on the larva at the point at which the ovipositor has been inserted. This character is more clearly discernible after the larva has formed into a puparium.

The maximum number of eggs deposited by a given female in confinement was 104 (see Table VI). The greater number of eggs is deposited during the first five or six days after the female emerges. They are usually deposited in large lots at intervals 24 to 48 hours apart, rather than in small numbers frequently. The greatest number of eggs deposited by a given female over a period of 24 hours was 62. No ovipositing female has been kept alive longer than 12 days. The great prolificness of this species is here seen from its habit of oviposition and in the proportion of sexes above mentioned. With an average life cycle of about 25 days, a deposition of from 90 to 100 eggs within the first five or six days of life, and the resulting progeny 75 per cent female, the multiplication is seen to be very rapid. Fruit-fly larvæ in the second instar may be successfully parasitized.

Adults fed with honey and water and confined in glass tubes held in partial darkness have been kept alive from two to three months without difficulty, provided no opportunity is given for oviposition. Of 274 adults emerging on September 3, 1917, one male lived for 61 days and one female for 69 days. Most of the males died between the ages of 30 and 50 days, while the majority of the females died between the ages of 40 and 60 days. Of 267 adults emerging on December 19, 1917, one male lived for 58 days and one female for 102 days. Nine of the females lived over 90 days. This long life was due to the mild temperatures of December, January, and February. Without food this parasite usually dies within 46 hours, although one individual has been held alive for 55 hours.

TABLE VI.—*Daily rate of oviposition of Tetrastichus giffardianus, 1916–17*

Date of oviposition.	Number of eggs deposited.														
	Female No.														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
October.															
9	30	....	34	....	....	....	....	....	....	....	....	....	....	....	....
10	....	53	....	....	10	....	9	12	8	7	14	....	....	....	....
11	12	6	30	51	24	52	20	7	....	3	1	....	....	....	....
12	22	7	....	23	(a)	7	4	7	7	....	10	....	....	....	....
13	....	....	....	....	....	....	....	21	10	8	1	....	....	....	....
14	10	8	....	....	....	....	13	4	15	....	4	....	....	....	....
15	....	....	....	10	....	20	....	1	....	10	....	....	....	....	....
16	....	15	9	....	....	....	....	(a)	8	8	....	....	....	....	....
17	(a)	....	12	....	....	(a)	....	....	....	5	(a)	....	....	....	....
18	....	....	(a)	(a)	....	....	....	....	....	(a)	....	....	....	....	....
20	....	15	....	....	....	....	....	....	....	....	....	....	....	....	....
21	....	(a)	....	....	....	....	....	....	....	....	....	....	....	....	....
23	....	....	....	....	....	....	(a)	....	(a)	....	....	....	....	....	....
July.															
27	....	....	....	....	....	....	....	....	....	....	....	....	2	24	61
29	....	....	....	....	....	....	....	....	....	....	....	....	53	24	....
30	....	....	....	....	....	....	....	....	....	....	....	62	7	10	7
31	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
August.															
1	....	....	....	....	....	....	....	....	....	....	....	....	(a)	....	25
2	....	....	....	....	....	....	....	....	....	....	....	....	....	7	....
4	....	....	....	....	....	....	....	....	....	....	....	(a)	....	....	....
5	....	....	....	....	....	....	....	....	....	....	....	....	....	2	2
7	....	....	....	....	....	....	....	....	....	....	....	....	....	(a)	(a)
12	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....
Total	74	104	85	84	34	79	46	52	48	41	30	62	62	67	95

a Died.

No. 1 to 6, inclusive, emerged on October 9, 1916.
No. 7 to 11, inclusive, emerged on October 10, 1916.
No. 12 to 15, inclusive, emerged on July 27, 1917.

PARASITISM OF THE MELON FLY BY FRUIT-FLY PARASITES

Though great quantities of melon flies, *Bactrocera cucurbitae* Coquillett, were reared in the laboratory from material collected about Honolulu during the years 1914, 1915, and 1916 after the fruit-fly parasites had become established, no parasites were reared from the material until September 4, 1917. On this date, 7 normal adults of *Tetrastichus giffardianus* were reared from a single melon-fly puparium. This puparium was part of a quantity secured from cucumbers collected in Honolulu two weeks previously. Before proceeding further it should be stated that a natural parasite of the melon fly was introduced into Hawaii by Mr. D. T. Fullaway in May, 1916. It was well established by the latter

part of 1917. The rearing of these 7 individuals of Tetrastichus from the melon-fly puparium was entirely owing to the parasitism of the host, while a larva, first by this natural parasite, *Opius fletcheri* Silvestri, and later by *T. giffardianus*. Proof of this is shown by the following results.

Prior to September, 1917, many attempts were made to rear the fruit-fly parasites from melon-fly puparia. No difficulty was experienced in inducing all of the species to oviposit in the larvæ, but all parasitized individuals developed into normal flies. The opiines were easily led to oviposit into melon-fly larvæ by transferring these larvæ from various vegetables to the fruits of *Mimusops elengi*, a fruit usually heavily infested with fruit-fly larvæ. There was no need for transferring the larvæ for *T. giffardianus*, however, as it readily entered any of the hosts of the melon fly, such as cucumber, pumpkin, etc., and quickly located and oviposited into the maggots. The interesting results of these artificial attempts to rear fruit-fly parasites from the melon fly may be briefly summarized as follows:

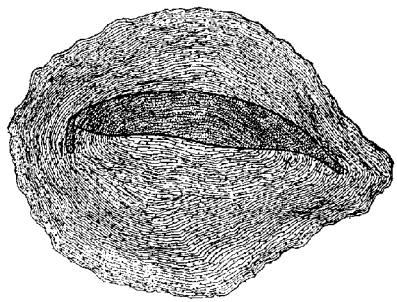

FIG. 34.—*Diachasma tryoni:* Dead encysted egg removed from melon-fly pupa. Length of capsule 0.55 mm.

During March, 1917, through the handling of melon-fly larvæ as described, a total of 154 eggs of *Diachasma tryoni* were deposited into 40 melon-fly larvæ, 17 eggs of *D. fullawayi* were deposited into 8 larvæ, 33 eggs of *Opius humilis* were placed in 8 larvæ, and 232 eggs of *Tetrastichus giffardianus* were deposited into 19 larvæ. All of these larvæ pupated normally. Some were mature when stung while others were only about two-thirds developed. From two to five days after each was stung, it was dissected, being now in the pupa stage, and the parasite eggs were counted and carefully examined. None of the opiine eggs hatched and only a small proportion of those of Tetrastichus. In every case, without exception, the opiine eggs developed only a little and then became closely encysted in a mass of transparent cellular material (fig. 34). The egg is at first only thinly surrounded with this encysting substance, but after two or three days it becomes densely inclosed, forming a homogeneous, ovoid body. The egg has then collapsed a little and has become brownish in color. It can be faintly distinguished as it lies in this body. The Tetrastichus eggs and some larvæ that managed to hatch became similarly encysted. In no case did a Tetrastichus larva live long

enough to ingest a visible quantity of food. As the Tetrastichus deposits its eggs in clusters of 8 or 10, they become "encapsulated" in such groups (fig. 35).

The presence of the eggs of these parasites in this dead, inclosed state offers no impediment to the normal development of the melon-fly larva to the adult fly. The adult emerges and carries within its body, usually in the fat masses, these eggs singly or in groups, until death. Death does not seem to be hastened by the presence of the foreign bodies within the body. On May 24 and 25, 1917, 25 melon-fly larvæ were stung by females of *Diachasma tryoni* and eggs deposited in each. The larvæ were then permitted to develop, pupate, and finally produce living adult flies. These flies were placed in large jars and kept alive for several months. On June 4, 14, and 20, July 10, August 10, and September 27, a few of these flies were killed and examined internally for evidences of the parasite eggs originally deposited in the larvæ. From 1 to 9 distinct, brownish, encysted eggs of *D. tryoni* were located in some portion of the body of each of the 25 flies on the foregoing dates.

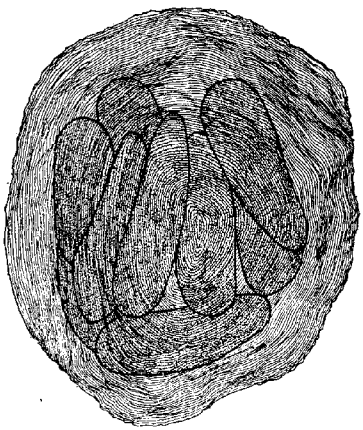

FIG. 35.—*Tetrastichus giffardianus:* Dead encysted cluster of eggs removed from melon-fly pupa. Greatest width of capsule 0.56 mm.

When, on September 4, 1917, as previously stated, 7 adults of *Tetrastichus giffardianus* were reared from a melon-fly puparium secured from the field, after such contradictory experiments had been carefully completed in March, renewed attempts were made to rear this species in the laboratory from the melon fly. The possible significance of the presence of the newly introduced melon-fly parasite, *Opius fletcheri*, was immediately recognized. As the host larva was powerless to resist the development of this parasite, it was assumed that a subsequent oviposition by *T. giffardianus* into larvæ previously stung by *O. fletcheri* might result in the rearing of adults of *T. giffardianus*. With this object in view, melon-fly larvæ were first subjected to attack by *O. fletcheri* and then by *T. giffardianus*. As the rearings and examinations from then on showed in every case where both species had parasitized the larvæ, the individuals of *T. giffardianus* developed and emerged as perfect parasites, and in every case in which only the latter oviposited

into the larva, no parasite developed and the fly matured and carried in its body the encysted eggs of *T. giffardianus*. In those cases in which both parasites oviposited, the larvæ of *O. fletcheri* always died while very young, and all of the larvæ of *T. giffardianus* survived. One case out of 201 occurred, however, wherein a female of *O. fletcheri* and 5 adults of *T. giffardianus* emerged from the same puparium. During September, October, and November, 1917, 3,092 adults of *T. giffardianus* were reared from 201 melon-fly puparia by the foregoing method of double parasitism. After all of the adults of *T. giffardianus* had emerged, microscopic examinations were made of the contents of each of the 201 empty puparia. In every case the dead larva of *O. fletcheri*, usually one to a puparium, was found. This was proof absolute that every host individual had been stung not only by *T. giffardianus* but also by *O. fletcheri*. In conjunction with this, 429 melon-fly larvæ were stung by *T. giffardianus* alone. These larvæ all matured to flies, and each of the flies when dissected was found to contain clusters of encysted dead eggs or larvæ of *T. giffardianus* in various parts of the body.

As the duration of the egg of *T. giffardianus* is shorter than is that of *O. fletcheri*, cases have been observed in which larvæ of the former were developing normally before the egg of the latter had hatched. This indicates that the immunization of the melon-fly larva against parasitic development is destroyed at the time it receives the egg of *O. fletcheri*.

This weakened resistance of the melon-fly larva to parasitic development is caused most probably by toxic substances injected into the larva by the female of *O. fletcheri* during the deposition of the egg. The reduced resistance of the larva is not caused by any mechanical injury or bacterial infection attending the insertion of the ovipositor. Larvæ have been pricked with fine cactus spines or heavily stung by *Diachasma tryoni* and then exposed to attack by *T. giffardianus*. In such cases the eggs of *T. giffardianus* invariably became encysted as usual and died. The eggs even became encysted in larvæ that had been almost killed by running a cactus spine through the body.

Unusual interest has attended the results of exposing melon-fly larvæ to the attack of *O. fletcheri*, followed by the exposure of the infested melon-fly larvæ to fruit-fly parasites other than *T. giffardianus*. In all such cases the fruit-fly parasite eggs failed to develop, becoming as tightly encysted as if the *O. fletcheri* egg had not been present. In other words, no fruit-fly parasites will develop in melon-fly larvæ under any conditions, except in the case of the eulophid *T. giffardianus*, and this species will do so only when combined with an egg or larva of the natural melon-fly parasite, *O. fletcheri*.

O. fletcheri will develop normally in fruit-fly larvæ. Laboratory experiments have proved this positively and it has been reared from fruit-fly puparia secured in the field in Hawaii on three separate occasions.

The parasitism of unnatural hosts has been investigated by others. Of Americans, the work of Mr. P. H. Timberlake is of unusual interest. He succeeded in inducing *Limnerium validum* Cresson, which is a natural parasite of the fall webworm (*Hyphantria cunea* Drury), to oviposit in caterpillars of the brown-tail moth (*Euproctis chrysorrhoea* Linnaeus), the gipsy moth (*Porthetria dispar* Linnaeus), and the rusty vaporer moth (*Notolophus antiquus* Linnaeus). In all the larvæ of *L. validum* failed to develop. Timberlake (7) states that the larvæ

FIG. 36.—*Galesus silvestrii*: Egg, 1 day old. Length 0.36 mm.

fail to survive the protective reactions of the host, which are visibly manifested by an accumulation of active blood cells or amœbocytes around the larvæ, the cast eggshells, and even the eggs themselves. The amœbocytes presumably attack the living eggs and larvæ, or at least ultimately efface the latter entirely.

RELATION OF THE INTRODUCED PUPAL PARASITES TO THE ESTABLISHED LARVAL PARASITES OF THE FRUIT FLY

GALESUS SILVESTRII

In addition to the larval parasites brought into Hawaii to control the Mediterranean fruit fly, a proctotrupid, *Galesus silvestrii* Kieffer, was introduced by Silvestri in May, 1913. This is a pupal parasite. Though readily breeding in fruit-fly puparia in confinement, it has never been established in the open.

Experiments with this species in the laboratory have shown definitely that it may act either as a primary or a secondary parasite, and that its tendencies are suspiciously more those of a secondary than of a primary parasite. Fruit-fly puparia containing developing larvæ of any of the opiine parasites above described or of *Tetrastichus giffardianus*, when exposed to attack by *G. silvestrii*, may produce adults of *G. silvestrii*. Extensive experiments with combinations of *G. silvestrii* and the larval parasites of the fruit fly have proved beyond doubt that a female of *G. silvestrii* will never place her egg loosely in a fruit-fly puparium if that puparium contains already the developing larva of some other parasite. In such cases she invariably feels about with her ovipositor until she locates the parasitic larva, into which she oviposits. This egg may hatch and ultimately a normal adult of *G. silvestrii* may emerge from the fully developed parasitic larva and bite its way out of the puparium.

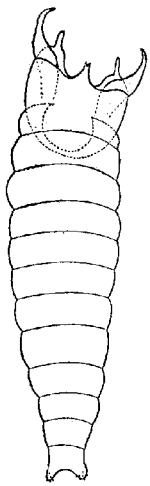

FIG. 37.—*Galesus silvestrii*: Newly hatched larva. Length 1 mm.

Before discussing the relation of *G. silvestrii* to the other fruit-fly parasites, a brief summary of its life history will be given.

Under ordinary circumstances the Galesus female attaches herself to the fruit-fly puparium (Pl. 32, E), inserts her ovipositor, and within 3 to 15 minutes places an egg into the fly pupa. The subelliptical egg (fig. 36) hatches in 2 to 3 days. The newly hatched larva (fig. 37) is strictly an internal feeder. Its general structure is strikingly like that of the newly hatched opiine larva. The heavily chitinized head, bearing long, curved mandibles, and the soft segmented body strongly suggest a close relationship to the opiines as shown in figures 3, 4, 23, 24, and 28. No tracheæ occur in the first-instar larva. With the molt to the second instar (fig. 38), the chitinized head is lost and the body is simple, without strong characters, and very much resembles the second-instar larvæ of the opiines. A third instar occurs, resembling the second. Whether or not there is an intermediate stage between the third and the last has not been determined. With the molt to the last larval instar a strong, well-defined, open tracheal system appears, with three pairs of large stigmata. The stigmata are borne on the first three segments back of the head. Heavily chitinized, sharply pointed mandibles are present (fig. 39). No waste material is voided by the larva. The midintestine is filled with this waste and is closed caudally. Just before pupation, in the prepupal stage, the entire waste accumulation is discharged. Into this meconium the exuvium of the mature larva is shed upon pupation. The pupa then lies with the caudal tip embedded in the voided meconial mass and the crumpled larval exuvium. The entire egg, larval, and pupal period averages, in Honolulu, from 25 to 32 days, depending upon the temperature. The adult emerges usually by pushing off the dorsocephalic cap of the puparium (fig. 40). Mating and oviposition occur immediately upon emergence. The female flies

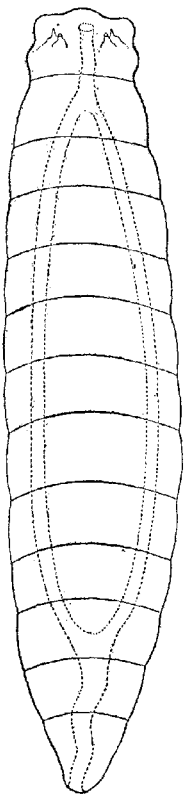

Fig. 38.—*Galesus silvestrii:* Larva of second instar. Length 1.5 mm.

Fig. 39.—*Galesus silvestrii:* Mandible of mature larva. Length 0.085 mm.

very little. It remains about the ground, entering crevices and crawling under various objects, apparently in search of fly puparia. In Hawaii it has been reared from the puparia of several muscid species, but it has never been reared from any material collected in the field. The female may reproduce parthenogenetically, the resulting progeny being males.

In the laboratory the female does not discriminate between parasitized and unparasitized fly puparia. She will insert her ovipositor into any puparium. If it contains only a developing fruit-fly pupa, the egg is deposited into the pupa. If the puparium contains a parasitic larva, which may be even one of the small Tetrastichus larvæ, her ovipositor will search out this larva and an egg will be deposited into it. A Galesus adult ultimately may mature within this larva. It will, of course, be dwarfed. These small adults are fertile. Such a habit indicates a strong hyperparasitic inclination.

During August, 1917, a total of 195 fruit-fly larvæ were parasitized in the laboratory by *Diachasma tryoni* or *Opius humilis*. These larvæ were then permitted to develop and to form into perfect puparia. The puparia were then immediately placed in cages containing females of *G. silvestrii* and left for 2 days. From 5 to 7 days later each puparium was opened and the contents examined. In every case the puparium contained a dead opiine larva or occasionally a pupa, in each of which was a rapidly developing, strong larva of *G. silvestrii*. This, then, was hyperparasitism pure and simple. To prove that these larvæ of *G. silvestrii* ultimately would develop to normal adults, a fresh lot of heavily parasitized fruit-fly larvæ was collected in the field. They were variously parasitized by *D. tryoni*, *D. fullawayi*, *O. humilis*, and *Tetrastichus giffardianus*, and some were unparasitized. The puparia thus formed from the larvæ were placed with 75 adults of *G. silvestrii* on September 26 and left for 24 hours. The puparia, 277 in number, were then removed and each placed separately in a small vial to await emergence. From this material 94 adults of *G. silvestrii* emerged, with both sexes well represented. Each of the 94 puparia producing the adults of *G. silvestrii* was then carefully dissected, opened, and examined. In 68 of the puparia the empty shell of a mature *D. tryoni* larva was found, and in each shell the cast mandibles of the larva of *G. silvestrii* as well as the characteristic prepupal meconial discharge of *G. silvestrii* was disclosed. In 3 puparia a similar larval shell of *O. humilis* was found, each with similar contents. In 1 puparium the shell remains of a mature *D. fullawayi* larva was found, and in it also were the larval mandibles and prepupal meconial discharge of *G. silvestrii*. In the remaining 22 puparia which had produced adults of *G. silvestrii* remains of the

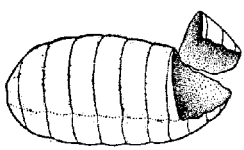

Fig. 40.—*Galesus silvestrii*: Fruit-fly puparium showing characteristic emergence hole made by adult parasite. Greatly enlarged.

fruit-fly pupa alone occurred, within which were the larval remains of *G. silvestrii*. The remaining 183, puparia not having been parasitized by *G. silvestrii*, produced 52 adults of *D. tryoni*, 14 of *O. humilis*, 1 of *D. fullawayi*, 12 of *T. giffardianus*, 3 fruit flies, and the balance dried up and produced nothing. Thus, in the 94 cases above cited, 72 occurred in which a normal adult of *G. silvestrii* emerged as a hyperparasite on an opiine parasite of the fruit fly. In 22 other cases it emerged as a normal primary parasite of the fruit fly.

As above mentioned, females of *G. silvestrii* will always oviposit into larvæ of *Tetrastichus giffardianus* as they lie developing in the fruit-fly puparium. *T. giffardianus* is a small parasite, being hardly one-half as large as *G. silvestrii*. Its larvæ are thus very small to receive the comparatively large egg of *G. silvestrii*. Larvæ of *T. giffardianus* about 0.7 mm. long are oviposited into by *G. silvestrii*, and the egg, which is about 0.35 mm. long, hatches and the larva of *G. silvestrii* develops, pupates, and may ultimately produce a dwarfed adult about 1.5 mm. long. The immature larva of *T. giffardianus* receiving the egg of *G. silvestrii* is able to develop to maturity. It is killed, however, before it is able to pupate. Females of *G. silvestrii* have actually oviposited into larvæ of *T. giffardianus* only 2 days old. In all of the experiments conducted to prove the hyperparasitic action of *G. silvestrii* over *T. giffardianus*, the eggs of the former were deposited into larvæ of the latter not more than 4 days old, the majority being only 2 and 3 days old.

On August 28 and 29, 1917, a total of 209 mature fruit-fly larvæ were exposed to attack by *T. giffardianus*. All of these larvæ formed into puparia during the next two days. On August 31 these 209 puparia were placed in a jar containing about 100 females of *G. silvestrii* and left until September 2. On September 5, 6, and 7, 59 of the puparia were opened, examined, and the contents carefully recorded. Each puparium contained from 3 to 19 well-developed larvæ of *T. giffardianus*, and in every case from 1 to 6 of these larvæ were dead and contained an active larva of *G. silvestrii* in the first, second, or third instar. The remaining 150 puparia were saved for adult emergences and ultimately produced fruit flies, adults of both species, and in some cases both species from the same puparium.

Thus we have positive proof that this proctotrupid may be strongly hyperparasitic upon any of the established parasites of the fruit fly that are now contributing toward its control in Hawaii, and that it may act with equal freedom as a primary parasite of the fruit fly.

PACHYCREPOIDEUS DUBIUS

A pteromalid, *Pachycrepoideus dubius* Ashmead, introduced from the Philippines by Mr. D. T. Fullaway in 1914 as a dung-fly parasite, has

been reared occasionally from fruit-fly puparia. It is not an important fruit-fly parasite, but the interesting relations that may exist between this and the other fruit-fly parasites has led to a laboratory study of this species, with results worth recording.

The female oviposits into the puparium, the egg being placed on the surface of the developing pupa within, and its larva develops on the pupa as an external feeder. Thus from the first it bears a well-developed tracheal system. The fly pupa is killed shortly after the parasitic larva hatches and the larva continues to feed upon the decomposing pupa. It feeds much in the sense of a scavenger. It does not consume all of the food in the puparium. The meconium is voided just before pupation and the pupa lies partly buried in the decayed remains of the fly pupa and its own meconium. The adult parasite emerges by cutting a small, circular hole in the puparium (fig. 41) and is immediately ready for mating and oviposition. Another minute meconium is discharged immediately upon emergence. The female may reproduce parthenogenetically with the resulting progeny all males. The entire period from deposition of egg to emergence of adult lasts only from 15 to 16 days.

FIG. 41.—*Pachycrepoideus dubius:* Fruit-fly puparium showing characteristic emergence hole made by adult parasite. Greatly enlarged.

As the larva of this parasite is an external feeder within the puparium from the first, and the larvæ of the other parasites are all internal feeders, the relationship between them is interesting. In a certain sense this parasite has been proved a primary, a secondary, or a tertiary parasite. As a primary it develops on the fruit-fly pupa, as a secondary it develops on larvæ or pupæ of *Tetrastichus giffardianus*, *Opius humilis*, *Diachasma tryoni*, *D. fullawayi*, or *Galesus silvestrii* as they occur as parasites in the fruit-fly puparium, and as a tertiary parasite it will of course develop on a larva of *G. silvestrii*, which in turn has been feeding in the larvæ of the above-mentioned opiines or *T. giffardianus*. In the laboratory, in large jars, this parasite indiscriminately oviposits into fruit-fly puparia to bring about all of the above conditions of parasitism and may be expected to act similarly in the field, when able to reach fruit-fly puparia. Fortunately it does not show any decided capacity for penetrating soil in search of fly puparia and is probably more attracted to situations harboring the dung-feeding species. The data demonstrating the various capabilities of this species as a primary, secondary, or tertiary parasite may be summarized as follows:

During November 24 and 25, 1917, a total of 341 fruit-fly puparia were secured from kamani fruits collected about Honolulu. These puparia were variously parasitized by *O. humilis*, *D. tryoni*, *D. fullawayi*, and *T. giffardianus*. On November 29 they were placed in a jar con-

taining about 100 females of *P. dubius* and left until December 2. They were then removed and on December 7 and 8 dissected and carefully examined. Thirty-one contained a dead larva or pupa of *D. tryoni* on which was feeding a mature larva of *P. dubius;* 73 contained a dead larva or pupa of *O. humilis* on which fed a larva of *P. dubius;* 2 contained a dead larva of *D. fullawayi* on which was a larva of *P. dubius;* 22 contained from 3 to 31 larvæ of *T. giffardianus*, some of which were in each case dead and on which fed a larva of *P. dubius;* 30 contained decomposed fruit-fly pupæ parasitized by *P. dubius* alone. Of the remaining 183 puparia, 46 were unparasitized, 88 were parasitized by only *O. humilis*, 31 by only *D. tryoni*, and 18 by only *T. giffardianus*.

Again, on October 23, 50 fruit-fly puparia containing developing larvæ of *O. humilis* and *D. tryoni* were exposed to attack by *G. silvestrii* for one day. On October 30 they were placed in a jar containing females of *P. dubius* and left for two days. They were then opened and examined on November 10 with the following interesting results: Thirty-five contained dead larvæ of *G. silvestrii* lying in dead larvæ of *O. humilis* or *D. tryoni*, on the whole of which was feeding in each case a larva of *P. dubius*.

The relation of the *P. dubius* to *T. giffardianus* is also of interest. Very frequently a puparium containing 15 or 20 larvæ or pupæ of *T. giffardianus* and exposed in the laboratory to attack by *P. dubius* will yield a normal adult of *P. dubius* and several normal adults of *T. giffardianus*. On November 23 and 24, 83 fruit-fly larvæ were parasitized by *T. giffardianus* in the laboratory. The 83 puparia forming from these were then placed with adults of *P. dubius* and left for three days. On December 8 the puparia were opened and examined. In 23 cases examined, from 4 to 20 dead larvæ of *T. giffardianus* were found, and on these in each case was feeding a mature larva of *P. dubius;* in 30 puparia from 1 to 10 living pupæ of *T. giffardianus* occurred together with from 3 to 20 dead larvæ of that species upon which were feeding larvæ of *P. dubius*, one in each instance; in the remaining 33 puparia from 7 to 19 individuals of *T. giffardianus* were developing in the absence of individuals of *P. dubius*.

In conclusion, it should be borne in mind that *G. silvestrii* is not known to be established in Hawaii as yet, and that *P. dubius* probably only parasitizes a fraction of 1 per cent of the fruit-fly puparia in the field, but that the relations between these and the other fruit-fly parasites, as detailed in the foregoing pages, can be expected as a natural sequence if they ultimately adapt themselves to Hawaiian conditions and become thoroughly established.

PHEIDOLE MEGACEPHALA AS A PREDACIOUS ENEMY OF THE FRUIT FLY

Although several species of ants are common in the Hawaiian Islands, the cosmopolitan ant *Pheidole megacephala* Fabricius greatly outnumbers all others and is enormously abundant throughout the islands at low elevations. It is quite probable that it checks and greatly limits the increase of several of these species. It is quickly drawn to any fresh or decaying animal matter and the soft body of the fruit-fly larva falls an easy prey to it. It has been generally assumed that this ant destroys large numbers of fruit-fly larvæ throughout the year. Very little definite data are on record, however, covering investigations on this relationship. Though the following records are not extensive, they all point in one direction and are significant.

Four hundred and eighty-eight kamani fruits collected from a certain tree in Honolulu on December 3, 1916, were spread out under the tree on a large piece of canvas covered with 1 inch of sand and left for 10 days. By this time practically all of the contained fruit-fly larvæ had developed and pupated in the sand beneath the fruits or had been carried away by ants. The sand was then sifted and the fruit-fly puparia were counted. A total of 606 fruit-fly puparia were obtained. This was an average of 1.2 larvæ per fruit. During November and December 969 more fruits were gathered from the same tree and taken to an ant-proof insectary to be held in boxes of sand for 10 days. From these 4,895 fruit-fly puparia were obtained, or an average of 5 larvæ per fruit. Here four times as many larvæ per fruit developed in the lot where the ants were excluded. From 1,301 fruits of the same kind collected from another tree in Honolulu in November, 1917, and similarly placed and held for 10 days on canvas under the tree, 3,742 fruit-fly puparia were ultimately obtained. From the same tree and collected at approximately the same date, 909 fruits were taken and brought to the ant-proof insectary. From these 10,119 fly puparia were secured. Here again almost four times as many larvæ per fruit were obtained from the fruits held away from the ants. On January 25, 1918, 664 loquats were collected from several trees in a certain yard in Honolulu and divided in half, one half going out on the canvas and the other part to the insectary. From the lot on the canvas, after 10 days' exposure, 156 fruit-fly puparia were gathered, and from those in the insectary a total of 840. In this case the ant-proofed lot produced over five times as many puparia. On January 29, February 2, and February 6, 1918, an aggregate of 3,100 fruits of the black myrobalan was collected from one tree. This total was divided in half as soon as each lot had been collected, the two portions being handled as in the above cases. From the fruit held in the insectary 6,668 fly puparia were obtained, while only 4,385 puparia were ultimately secured from the half placed out on

the canvas. Here the lot held away from the ants was 1½ times as great as that exposed to the ants.

The foregoing should represent a very conservative estimate of the destruction of fruit-fly larvæ by this ant. It shows consistently that from one-third to four-fifths of the larvæ developing in all fruits in the field very probably never mature to adult flies.

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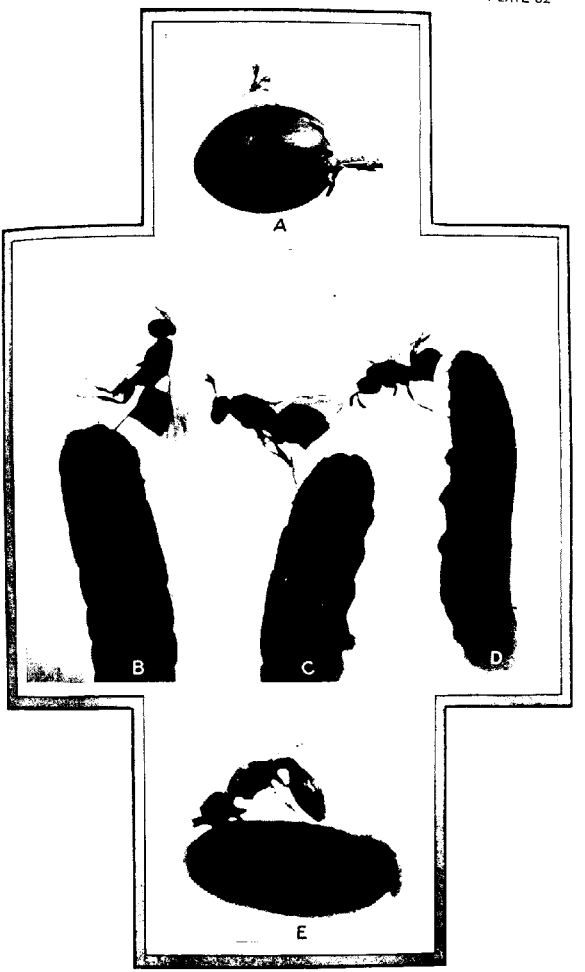
PLATE 32

Oviposition of fruit-fly parasites:

A.—*Diachasma tryoni* ovipositing into fruit-fly larva in fruit of *Mimusops elengi*.
B, C, D.—*Tetrastichus giffardianus* ovipositing into fruit-fly larva.
E.—*Galesus silvestrii* ovipositing into fruit-fly puparium.


A
B
C
D
E

OBSERVATIONS AND EXPERIMENTS ON INTESTINAL TRICHINÆ

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SCOPE OF WORK

Although *Trichinella spiralis* has been studied by many investigators, the literature on the subject is almost exclusively devoted to the morphology and life history of the parasites and their relation to the disease which they produce in man and in other mammals. Several investigators have recorded some casual observations on the physiology of these parasites, particularly with reference to the resistance of the encysted larvæ to unfavorable conditions. No attempt has heretofore been made, however, to present a systematic account of the behavior of these organisms under the influence of various physical and chemical stimuli at different stages of their life history. Recently Ransom (*6*)[1] in the course of a report of an investigation on the effects of refrigeration on the encysted larvæ of *Trichinella spiralis* has presented some interesting data relative to their behavior when freed from their cysts by artificial digestion. Ransom and the present writer have continued these investigations and have obtained a considerable amount of data[2] on the resistance of the larvæ to high temperatures and to various physical and chemical agents.

Inasmuch as little has been known heretofore concerning the physiological processes of the parasites concomitant with their growth in the small intestine, it appeared to the writer that observations and experiments on the intestinal forms, with special reference to their behavior under various conditions, might yield some useful information. The present paper embodies the results of this work and also includes observations on the molting of the larvæ *in vitro*, a phenomenon which, so far as the writer is aware, has not been hitherto recorded.

METHODS OF STUDY

In nearly all the observations and experiments recorded in this paper the parasites were obtained from albino and hooded rats which were artificially infected by feeding ground-up trichinous pork. It was found that after being kept on a vegetable diet for several days, rats devour meat very readily even though it is badly decayed. In some respects

[1] Reference is made by number (italic) to "Literature cited," p. 482.

[2] These results have not yet been published.

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rabbits and guinea-pigs are less favorable hosts from the experimenter's point of view. They will not swallow meat unless it is fed forcibly. Even under these conditions the attempted infection is not always successful in the case of rabbits, and occasionally yields negative results in guinea-pigs. The former were observed to regurgitate meat several minutes after being fed. However, aside from these considerations the size of the intestine and the character of its contents make it rather difficult to isolate the parasites from the intestine of rabbits and guinea-pigs. The intestine of the rat, being considerably smaller, presents the parasites in greater concentration. In the writer's experience the contents of the intestine of a rat even a few hours after a meal do not seriously interfere with the detection and isolation of the parasites. However, when it is desired to keep the host animals alive for several weeks, rats are not to be relied upon unless they are only lightly infested with the trichinæ and are kept in a warm place and protected from drafts. A heavy invasion of trichinæ sets up an acute intestinal inflammation in rats and causes their death as early as the third day after artificial infection. A sudden change in temperature or a draft is very likely to bring them down with pneumonia. If it becomes necessary, therefore, to keep the hosts alive for a month or longer, rabbits or guinea-pigs should be used in preference to rats. Instead of attempting to feed the meat forcibly to these animals, the writer found it more desirable to use the following procedure: The meat is thoroughly chopped up and then digested in an artificial gastric juice for about 18 hours at a temperature of 38° to 40° C. The formula for artificial gastric juice recommended by Ransom (6) has proved entirely satisfactory except that it was found that by reducing the amount of sodium chlorid to 2 grams per 1,000 cubic centimeters of fluid, a more thorough digestion of the meat was obtained.[1] After digestion of the meat the fluid is slowly decanted and replaced by a 0.7 per cent solution of sodium chlorid. The parasites are allowed to settle to the bottom, and the supernatant fluid is again decanted. This may be repeated until the parasites have been thoroughly washed. They may then be transferred to a smaller vessel and taken up in a fine pipette with some of the salt solution and forced down the esophagus of rabbits or guinea-pigs. This procedure has invariably resulted in a successful infection.

Intestinal trichinæ were obtained from their hosts as follows: After the animal had been killed by chloroform, the intestine was removed to a large petri dish containing a 0.7 per cent solution of sodium chlorid. The intestine was then slit open from end to end and the mucosa carefully scraped with the dull edge of a scalpel in order to remove the parasites from their places of attachment. They were then picked out with the aid of a dissecting microscope by means of a fine pipette, the opening

[1] The modified formula is as follows: Water, 1,000 cc.; hydrochloric acid (sp. gr. 1.19), 10 cc.; scale pepsin (U. S. P.), 2.5 gm.; sodium chlorid, 2 gm.

of which was too small to admit the coarser intestinal débris. The organisms were transferred to a 0.7 per cent solution of sodium chlorid in a watch glass which could be mounted on the stage of the microscope in order to make observations. To make out details slide preparations were made. When it was desired to keep the parasites in the laboratory overnight they were transferred by means of the pipette to a second watch glass in order to get rid of as much intestinal débris as possible. If this precaution is not taken putrefaction may set in, which, besides causing an offensive odor, forms a film on the surface and interferes with the observations.

Unless otherwise stated the parasites were kept in a 0.7 per cent solution of sodium chlorid which will be referred to as a physiological salt solution. In the description of the experiments and observations reference is made to the time which elapsed between the feeding of the trichinous meat to the host and the isolation of the parasites from the intestine. Inasmuch as albino rats do not as a rule eat the meat as soon as it is offered to them allowance must be made for the intervening period, which may vary from a few minutes to several hours.

MIGRATION OF THE LARVÆ FROM THE STOMACH TO THE INTESTINE

When trichinous meat is fed to a suitable host the cysts are digested in the stomach, the larvæ becoming free in the lumen. The following observations point to the conclusion that the decapsuled larvæ do not linger in the stomach but make their way into the small intestine very promptly after their liberation from the cysts. Furthermore, to judge from their behavior *in vitro*, it appears highly probable that their own muscular movements aid them in this change of location.

When the stomach still contains trichinous meat in various stages of digestion few decapsuled larvæ are found in it, although the small intestine may contain many newly arrived parasites. The latter may still be tightly coiled and, therefore, probably unattached to the mucosa. Within four hours after feeding trichinous meat, a number of tightly coiled larvæ were found in the intestine, but none were seen in the stomach. Eighteen hours after feeding, the small intestine was found to contain numerous parasites, some attached to the mucosa and active, others still unattached and coiled, the latter lying free in the lumen. The stomach contents of such hosts were usually found to be free from parasites. Feeding trichinous meat in which the parasites are no longer alive or so reduced in vitality as a result of refrigeration or other means of attenuation that they fail to survive the action of the gastric juice, does not result in a rapid transfer of the larvæ from the stomach into the intestine. Within about 18 hours after feeding such meat numerous larvæ were found in the stomach. The parasites were uncoiled and inactive and somewhat paler than larvæ known to be alive. In the small intestine the parasites were found in various stages of digestion.

Ransom (6) has shown that after trichinous meat has been digested at a temperature of 37° to 40° C. in an acidified solution of scale pepsin the parasites are liberated from their cysts. When taken out of the incubator the larvæ in the artificial gastric juice are found to be very active. Their activities are due to vigorous muscular movements, which propel the parasites and are thus locomotory in nature. Assuming that after liberation from their cysts in the stomach of a rat or other host the larvæ exhibit the same activity, it is probable that their own movements hasten and guide their transfer to the intestine. Chemotactic influences may play an important part in the migration of the larvæ from the stomach to the intestine.

The nonliving larvæ apparently remain in the lumen of the stomach until they are forced into the intestines with the chyle.

EFFECTS OF GASTRIC DIGESTION ON THE LARVÆ

Ransom (6) found that after artificial digestion the decapsuled larvæ may be kept active for over three weeks in a physiological salt solution. Ransom and the writer kept decapsuled larvæ alive in Ringer's fluid at a temperature of about 10° C. for seven weeks, at the end of which period they showed activity on a warm stage.[1] At body temperature the life of the decapsuled larvæ kept *in vitro* is brief according to the writer's observations, seldom exceeding 48 hours. But whether the larvæ survive several weeks or several hours, they neither increase in size nor exhibit any other morphological changes which can be attributed to growth. After artificial digestion they can maintain their vitality outside of the host for varying periods, depending on the temperature and osmotic pressure of the medium in which they are kept, but they do not resume their processes of growth and morphogenesis, which came to a halt coincident with encystment.

The question arises whether natural gastric digestion not only releases the parasites from their cysts, as artificial digestion does, but also in some way stimulates them to growth and development. That is, may not the impulse which leads to growth originate in the stomach of the host, the intestine merely supplying a suitable environment for continuation of the process begun in the stomach? Within four hours after feeding trichinous meat the writer isolated several larvæ from the small intestine. They were tightly coiled and apparently quite unaffected by their passage through the stomach and brief sojourn in the intestine. After being transferred to a physiological salt solution they were placed in a refrigerator at 10° C. They remained tightly coiled in the same way as larvæ obtained by artificial digestion.

Coiled trichinæ obtained from the intestine 15 hours, 18 hours, and 24 hours after feeding trichinous meat were similarly isolated and kept

[1] The results of these experiments have not yet been published.

alive in a physiological salt solution for a number of days without their undergoing any growth changes. These larvæ must have been in the small intestine for at least several hours, yet their larval condition remained unaltered and they were capable of living for several days in a salt solution. In view of these facts and in the light of what will presently be described with reference to the behavior of the intestinal parasites which have begun further development, it is apparent that the effects of natural gastric digestion on the larvæ is very much like the effects of artificial digestion; that is, the parasites are liberated from their cysts, but are not stimulated to further development.

CHANGES IN THE BEHAVIOR OF THE DEVELOPING LARVÆ

As has already been stated, larvæ taken from an artificial digestive medium after a period of incubation of 18 hours are quite active. Even after being washed in a physiological salt solution several times, the parasites still exhibit movements at room temperature. Gradually they become sluggish, the posterior end of the worm ceasing its contractile movements entirely, while the anterior end may still exhibit feeble activity. The worms finally coil up tightly and lapse into a quiescent condition, which characterizes these parasites in their normal locations in the voluntary muscles of the host.

In comparison with the quiescent state of the intact larvæ at room temperature the sexually mature and the maturing parasites taken from the small intestine and kept under observation in a physiological salt solution present a marked contrast. The intestinal forms continue their movements with almost unabated vigor until they succumb. Their activities are not as feverish as those of the intact larvæ which have been artificially stimulated, but are more persistent, more regular, and independent to a considerable extent of any external stimulation. Under none of the conditions to which they were subjected have the intestinal trichinæ been observed to coil up. Not even the depressing temperature of a refrigerator (10° C.) caused them to manifest the least traces of becoming coiled. The cold rendered them temporarily rigid, but they remained either completely elongated or exhibited a sinuous outline. It was quite evident that the low temperature paralyzed their movements, leaving each individual in the posture which it had assumed prior to coming under the influence of the cold.

It appears, therefore, that in addition to certain morphological differences the intestinal forms may be readily differentiated from the intact larvæ by their behavior *in vitro*. This is not the only physiological criterion, however, which can be employed to distinguish the maturing forms from the encysted larvæ. The latter have been known for a long time to have marked powers of resisting various toxic agents, in which respect they also stand out in sharp contrast with the developing forms,

as will be shown elsewhere in this paper. The last criterion can therefore be employed as a check on the first to distinguish the early developing intestinal larvæ from the intact forms, particularly when the morphological state following growth in the intestine is not yet sufficiently advanced to warrant a diagnosis on the basis of structure.

In order to determine whether the change in the behavior of the larvæ becomes established coincident with the resumption of growth in the small intestine, the organisms were taken from their hosts within 18 to 24 hours after feeding trichinous meat. Upon examination some of the parasites were seen to be in process of molting, and their subsequent behavior at room temperature showed that individuals which have reached the molting stage have abandoned the larval habit of becoming coiled. Of the nonmolting larvæ, some continued their active movements, whereas others became sluggish and finally coiled up. A number of active forms molted while they were kept under observation, and many of the molting as well as the nonmolting forms succumbed within a few hours. The quiescent forms which became coiled neither resumed their activities spontaneously nor perished at room temperature. Some of these forms were stored in a refrigerator where they maintained their vitality for many days. In fact, they behaved in the same way as larvæ obtained by artificial digestion of trichinous meat.

In view of the fact that certain larvæ obtained from the host within 18 to 24 hours after feeding trichinous meat may already have molted at least once in the intestine, it is safe to assume that the active larvæ which did not molt while they were kept under observation had already cast off their cuticles in the intestine of the host. At least their behavior *in vitro* showed that they are to be classed with the actively molting forms.

These observations show that larvæ which are in the act of molting, as well as larvæ which have molted prior to their isolation from the intestine, have lost the power of assuming the tightly coiled posture which characterizes the intact larvæ. They continue their movements until they succumb, and exhibit other traits characteristic of larvæ which are definitely known to have molted. Thus they may undergo rapid disintegration when kept in a physiological salt solution at room temperature and exhibit an intolerance for certain toxic substances, which will be discussed elsewhere in this paper.

MOLTING OUTSIDE OF THE HOST

Larvæ taken from the intestine within 18 hours after feeding trichinous meat do not as a rule show any evidence of having molted. If the individuals that continue their movements one or two hours after isolation from the small intestine are carefully observed, the molting process may be studied from its initial stage to its completion. The first evidence is

a retraction of the larva from its cuticle, leaving the latter empty at one end. This is usually observed to occur first at the posterior end. The empty portion continues to increase in size, and gradually the anterior end of the worm begins to contract in the same manner as the posterior end. The worm now assumes the appearance of being incased in a cuticle which is a little too long for it. The head end usually breaks through the membrane and continues lively movements until the entire worm pulls out, leaving the cast-off skin behind.

It is a significant fact that certain larvæ isolated from the small intestine as late as 24 hours after feeding the host trichinous meat resume their coiled-up larval posture and do not undergo any further changes when kept in physiological salt solution, whereas others under the same conditions continue their lively movements and undergo a molt. The active larvæ have apparently been stimulated by some factors present in the small intestine. Their greater activity at room temperature as compared with that of intact larvæ is evidence that their metabolism has been accelerated, and that this change in the rate of metabolism is nonreversible is evident from the fact that the parasites no longer react in the former manner to a lowering of the temperature, but continue active. Their subsequent molting would indicate that the influences under which the parasites resume their processes of growth and morphogenesis are so strong that having once been initiated the reaction proceeds even though the environment under which it normally occurs is replaced by an inert solution of sodium chlorid. The decapsuled larvæ prior to their stimulation in the small intestine are in a condition of physiological stability. They may, of course, be artificially stimulated to abnormal activities by various physical and chemical agents which if of sufficient intensity and duration soon result in their total destruction. But when the larvæ are mildly stimulated, the reactions usually subside shortly after the exciting cause is removed, and the larvæ again lapse into a quiescent state. Artificial physical and chemical stimuli, short of those that destroy the life of the parasites therefore produce reversible reactions. Under the influence of conditions to which the larvæ are subjected after reaching the small intestine of the host animal, a series of reactions is initiated which produce morphological changes in the parasites, leading toward their sexual maturity. These reactions are nonreversible, as they continue for a relatively long period of time in the absence of conditions that brought them about.

The molting process in the larvæ proceeds rather slowly at room temperature. At a lower temperature it may be suppressed, whereas at body temperature it may be greatly accelerated, as the following experiment will show:

Larvæ were obtained from a rat within 24 hours after feeding trichinous meat. A few parasites were placed in each of three watch glasses containing a physiological salt solution, and kept, respectively, in an incu-

bator at 40° C., in a refrigerator at 10° C., and at room temperature. At the end of about three hours each watch glass was examined and the following results noted: The larvæ from the refrigerator were rigid though not tightly coiled. After standing at room temperature for several minutes they became active, but no evidence of molting could be found. A careful examination of the contents of the watch glass failed to show any cast-off cuticles. The larvæ which had been left at room temperature were in the act of molting, but with the possible exception of two or three, the process had not been completed. The larvæ from the incubator were feverishly active and nearly all of them had completely cast off their cuticles, which were found at the bottom of the dish. Some were in the act of molting again.

Thus it is evident that temperature is a prime factor in the growth of the intestinal trichinæ. It is rather significant that a temperature of 10° C. inhibited the growth process without exerting a deleterious effect on the larvæ. In fact, after removal to room temperature one of the larvæ which had been kept in the refrigerator was seen to commence to molt, but as the observation was not continued it is not known whether the process was completed. The life processes of *Trichinella spiralis* under experimental conditions are modifiable to a considerable extent by certain environmental factors. Within certain limits they may be retarded by low temperatures and accelerated by high temperatures after the parasites are taken out of their host.

In the above experiment it was noted that certain larvæ behaved quite differently from the others. At room temperature as well as in the incubator they remained tightly coiled. The parasites in the dish taken from the incubator were all active at first, but at the end of about 30 minutes a number of larvæ became sluggish and finally coiled up. The assumption was made that the coiled larvæ were still intact so far as further growth was concerned and that they had apparently remained uninfluenced by their stay in the intestine. It was obvious that the incubator temperature did not stimulate them to development, but in order to settle this point more definitely the following experiment was performed.

A rat was killed within 20 hours after feeding trichinous meat. The larvæ after being isolated from the intestine were allowed to remain at room temperature for about an hour and only those which became tightly coiled were isolated and placed in another watch glass. The latter was kept under observation for about 30 minutes, and those larvæ which were not definitely coiled were taken out by means of a pipette. The dish was then covered and placed in an incubator at 40° C. for three hours. When the parasites were removed to room temperature they were quite active. Gradually their activities ceased, and they began to coil up. No cast-off cuticles were found in the dish and no parasites were seen to be molting.

It would therefore seem that it is not the high temperature which is responsible for the rapid molting of the larvæ, but that it merely hastens the process commenced in the small intestine. Larvæ which by their behavior appear to have escaped the intestinal influences do not molt even under a favorable temperature.

SURVIVAL OF INTESTINAL TRICHINÆ OUTSIDE OF THE HOST

One-day-old intestinal trichinæ are sensitive to the sudden change of environment and die within a few hours after their transfer from the intestine to physiological salt solution, either by a gradual process of disintegration in which case there is a dissolution of the worm progressing from one end to the other, or else they become rigid and begin to undergo granular degeneration without dissolution. It was found that such parasites do not even survive for 24 hours in a refrigerator at a temperature of about 10° C. Two-day-old intestinal trichinæ are not quite so sensitive and may be kept alive for 24 hours at 10° C. but not much longer. In 3-day-old intestinal parasites the resistance is greater. They have been kept alive in a refrigerator for two days, though at room temperature they succumbed earlier. Four-day-old and older intestinal trichinæ may be kept alive without difficulty for several days at a low temperature. Thus after development has actually begun in the intestine the tolerance to unfavorable conditions increases in proportion to the age of the parasite. The larvæ before they have been affected by their presence in the intestine are highly resistant to unfavorable conditions. On the other hand the youngest intestinal forms, shortly after the resumption of growth and morphogenesis, can not withstand abrupt changes which interfere with their developmental processes. The sexually mature forms again pass into a condition of relative stability which is accompanied by an increase in the power of resistance to unfavorable surroundings.

These observations are in harmony with the observations on the survival of the intestinal trichinæ after the death of their host. The writer found that within about 12 hours after the death of the host 1- or 2-day-old intestinal trichinæ usually perish, though occasionally a few may remain alive. They lose their hold on the mucosa and lie free in the lumen of the intestine where they undergo granular degeneration. Older intestinal trichinæ survive for a longer period, since they have been found still alive after the host had already begun to undergo decided putrefactive changes.

SPONTANEOUS DISINTEGRATION OF INTESTINAL LARVÆ IN VITRO

In the course of the observations on the parasites removed from the intestine after artificial infection it was noted that when the larvæ are kept in salt solution at room temperature some of them begin to disintegrate spontaneously. In fact the writer observed on several occasions

that watch glasses which but two hours earlier contained numerous larvæ had become almost free from the worms. This phenomenon was so striking that it was studied in detail as to the method of occurrence. The worms utilized in these observations were obtained from the host 24 hours after artificial infection. For the first 2 or 3 hours after the larvæ were isolated and kept at room temperature, the phenomenon was but seldom observed. Later, epidemics of disintegrations were noted, and worms at various stages of degeneration were readily found. The first sign of the process is a granulation of the worm at one end, more often at the anterior end. This is followed by a disappearance of the granules, the worm becoming gradually smaller. Occasionally the writer noted parasites in which the two ends had degenerated and the middle part was still intact. The wave of disintegration spreads slowly, and involves not only the internal organs, but the cuticle as well. In this respect, as will be shown later, the process differs from disintegration induced by potassium cyanid.

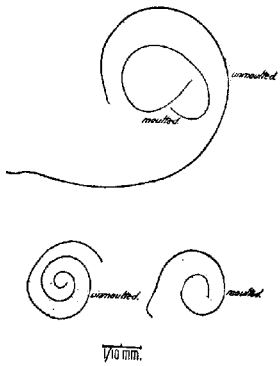

FIG. 1.—Skeleton outlines of two dwarfed trichinæ after the first molt outside of the host and of two unmolted larvæ from the same host about 18 hours after artificial infection.

The writer was at first inclined to believe that the absence of the intestinal contents of the host was perhaps responsible for the degenerative changes of the worms. However, even when the worms were kept in salt solution to which the intestinal contents of the host were added the process still occurred.

Several interesting speculations suggest themselves in this connection. The degeneration may perhaps be associated with a failure to undergo successful molting. It is not at all improbable that worms which have been stimulated by certain factors of the intestinal environment should, after sudden removal from that environment, undergo degenerative changes. There is a further possibility that the lack of proper food may check the impulse to growth and development, and lead to the process of degeneration. Finally, it is possible that concomitant with the growth of these parasites they liberate toxic substances which in an artificial environment exert a deleterious effect upon the parasites themselves. The last hypothesis can be subjected to experimental verification and the writer hopes to study this phase of the subject in connection with studies which are now in progress on the possible presence of toxic substances in the sera of hosts harboring trichinæ. It may be mentioned that Tallqvist (7) found in *Bothriocephalus latus* a proteolytic ferment which was capable of destroying the proglottids of that tapeworm.

DWARFS PRODUCED IN CULTURE DISHES

In the course of the observations on the molting of the larvæ in culture dishes it was found that sometimes after the first molt the parasite is only a little more than one-half the size of the unmolted larva (fig. 1, 3). These dwarfs maintain their vitality for some time after the molt, although they appear to be exceedingly sluggish. Their tissues, too, are not as transparent as the tissues of normal larvæ after the first molt. In fact the worm as a whole is darker in color and presents a very compact appearance. It is possible that the decrease in size is a result of food deprivation involved in the removal of the parasites from their normal environment to a culture dish. It is rather remarkable that under such conditions a second molt should be initiated almost immediately after the casting off of the first cuticle. The outline of the worm shown in figure 3 is of a dwarf larva of *Trichinella spiralis* in the course of the second molt. The parasite was obtained 18 hours after artificial infection, and the observation was made about 4 hours after the worm had been kept at room temperature.

FIG. 2.—*Trichina spiralis:* Outline drawing of a larva in the act of molting outside of the host.

CHANGE IN RESISTANCE OF INTESTINAL TRICHINÆ

A number of investigators have been struck by the marked powers of the intact larvæ to resist various unfavorable conditions. Davaine (3) states that he kept larvæ isolated from the muscles alive in fresh water for a month. The same writer also notes that after having reached the adult stage in the intestine this resistance is lost, and that the worms perish in fresh water within one hour.

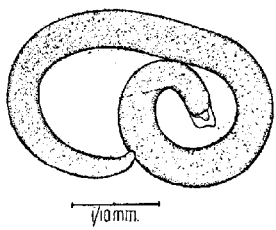

FIG. 3.—*Trichina spiralis:* Outline drawing of a dwarf larva after the first molt outside of the host. The beginning of the second molt is indicated.

The present writer has observed that the decapsuled larvæ are very tolerant to distilled water, concentrated sodium-chlorid solutions, weak solutions of alcohol (35 per cent), potassium cyanid, acids, and many other toxic agents. After the first molt as well as after subsequent molts, and to a lesser extent while the molt is in progress, the tolerance of the larvæ is suddenly reversed and is replaced by a marked sensitiveness. Distilled water is highly toxic to them and brings about their death in a few minutes. The parasites have lost their tolerance for acid, and succumb very rapidly to very weak dilutions (999 cc. of water and 1 cc. of hydrochloric acid, sp. gr. 1.19) This marked sensitiveness character-

izes the intestinal forms at the various stages of their development. The effects of potassium cyanid on the intact larvæ and on the molting forms have been studied especially with a view of determining changes in the resistance of the parasites at various stages of their life history.

These studies have yielded interesting data which the writer expects to present in a separate paper. The poison causes a disintegration in the tissues of the worms without influencing their general outline, due undoubtedly to the cuticle remaining unaffected. Moreover, the worms exhibit a differential sensibility to the poison along the major axis, the wave of disintegration in the intact decapsuled larvæ starting at the anterior end and proceeding posteriorly. When the head end shows a marked paling of the protoplasm accompanied by the beginning of granular degeneration the posterior end of the worm is normal as to color and transparency. The maturing larvæ as well as the sexually ripe specimens taken from the small intestine are not only more susceptible to potassium cyanid and perish almost instantaneously in dilutions in which the decapsuled larvæ survive for several hours, but show a complete reversal of axial susceptibility by being affected in the posterior end, the wave of disintegration gradually moving toward the anterior region. In *Trichinella spiralis* the posterior region consists largely of reproductive cells which, as growth takes place in the small intestine, divide very rapidly and produce thousands of germ cells. In fact, the development of the larvæ in the small intestine is essentially a sexual metamorphosis characterized by a rapid division of the germ cells and the maturation of the latter, accompanied by the appearance of the accessory reproductive organs. It is rather significant that while these changes are taking place in the larvæ the axial susceptibility of the worms to such a depressing agent as potassium cyanid should show the decided reversal described above. In the writer's opinion these facts are a striking confirmation of the views expressed by Child—namely, that there is a direct relation between susceptibility to depressing influences and the degree of metabolic activity.[1]

How can the sudden change in susceptibility of the worms to toxic agents be accounted for? In view of the fact that even the intact larvæ exhibit a differential sensibility to potassium cyanid along their major axis, the cuticle as the all-important factor underlying the resistance of the worms to toxic substances must be eliminated. The fact that the disintegration of the parasites under the influence of potassium cyanid is not haphazard but follows a definite course is direct evidence that the disintegration is a reaction between the tissues and the poison independent of the permeability of the cuticle, and that the rate of reaction varies in different regions of the worm. The reversal of susceptibility in the

[1] Child has published numerous papers dealing with this problem. His views are summarized in two books cited in the list of references (*1*, *2*).

intestina forms is a further confirmation of the view that the tissues of the organism are not passive but take an active part in the reaction. The increased susceptibility of the intestinal worms to potassium cyanid is also probably independent of the cuticle since this increased susceptibility expresses itself in many other ways which are more or less independent of permeability of the membranes of the worms to substances in solution. The loss of tolerance of the intestinal worms to cold, their failure to survive outside of the host for any length of time, their sudden disintegration in salt solution, and the gradual reappearance, though to a markedly lesser degree, of their tolerance to various conditions would indicate that the differences in susceptibility between the intact larvæ and the rapidly developing forms are due to a complete reorganization of the latter as a result of the resumption of differentiation and development.

The encysted larvæ are quite inactive under normal conditions. They neither increase nor decrease in size, but maintain a morphological balance which must have its basis in a delicate adjustment between their nutritive income and their outgo as a result of their processes of oxidation and excretion. With the resumption of growth and further differentiation in the small intestine, the physiological adjustment between income and outgo rapidly breaks down, their constructive metabolism running far ahead of the destructive processes. This leads to an increase in size of the animal and to a differentiation of organs not developed in the encysted larvæ. The intestinal forms in the early stages are thus characterized by a period of rapid growth and may, therefore, be regarded as physiologically younger than the encysted forms. Under the intestinal influences they experience a rejuvenescence, exhibiting a high metabolism which decreases again with age. Parallel with their aging they become more tolerant to adverse conditions. In other words, in the intestine the larvæ recommence a typical life cycle, marked at first by a period of rapid growth and an extreme sensitiveness to unfavorable external conditions, and followed by a period of relative stability and gradually by a reduction in susceptibility to adverse influences.

ATTEMPTS TO INDUCE MOLTING IN THE LARVÆ ARTIFICIALLY FREED FROM THEIR CAPSULES

Trichinella spiralis is particularly remarkable in its lack of host specificity. It is capable of reaching maturity in almost any mammalian host and is even able to develop in the intestine of birds. This adaptability to a varied environment would indicate that the factors favorable for its development are the general conditions prevailing in the intestine of warm-blooded vertebrates, such as temperature, food, alkalinity, etc. It therefore appeared to the writer that it would not be a hopeless task to attempt to induce the larvæ isolated from their cysts to molt on artificial media. Thus far all efforts have proved futile, but the work is being

continued with the hope that a medium may be found in which the larvæ will be capable of undergoing the molt that normally occurs a short time after they reach the intestine in the usual course of events when they are swallowed.

In looking through the literature relating to trichinosis the writer has found but scant references to experiments on the possibility of causing the development of the decapsuled larvæ of *Trichinella spiralis in vitro*. Fiedler (*4*) placed pieces of trichinous meat in the gut of a freshly killed rabbit. He tied the gut at both ends and placed it in water at a temperature of 25° to 28° R. Within five days the gut was opened but the trichinæ appeared to be unaffected. Pagenstecher (*5*) states that he did not succeed in causing *T. spiralis* to develop by means of artificial gastric juice. This investigator also placed a portion of intestine containing trichinæ in a sugar solution at body temperature for 24 hours, which resulted in the death of the worms. Davaine (*3*) states that he convinced himself that trichinæ do not develop outside of the host in water or in any other medium.

Among the media tried by the writer the following may be mentioned: Pure blood serum (of rodents), blood serum diluted with salt solution, pancreatin dissolved in an alkaline medium, neutral broth, alkaline broth and various sugar broths, mixtures of pancreatin with various broths, and contents from the small intestine mixed with salt solution. The parasites obtained from trichinous meat by artificial digestion were washed in salt solution and in weak alcohol in order to eliminate bacterial decomposition of the media. The latter were inoculated with the parasites taken up in a sterile pipette with a little sterile salt solution and then placed in an incubator at body temperature. The most favorable results have been obtained with mixtures of blood serum and salt solution and with dextrose broth. In the serum and serum dilutions the larvæ lived considerably longer than when kept in physiological salt solution at incubator temperature, exhibited marked activities, and in one case there were indications of molting. Certain changes in structure were noted which can not be definitely described. The cuticle at the anterior end of the worm had entirely disappeared.

In dextrose broth the larvæ were still alive and active after 24 hours at a temperature of 38° C., and continued alive for two days at room temperature, whereas in plain broth and in other sugar broths, as well as in neutral media, such as various salt solutions, they died before 24 hours had elapsed. In one case larvæ kept in an alkaline pancreatin solution were found to exhibit unusual acivity, including intestinal peristalsis.

SUMMARY AND CONCLUSIONS

(1) The larvæ of *Trichinella spiralis* do not linger in the stomach of the host after they are freed from their capsules, but pass into the small intestine.

(2) The passage of the larvæ through the stomach does not stimulate them to further growth and development, and a brief sojourn in the intestine is insufficient to initiate those processes which lead to sexual maturity.

(3) Larvæ from the intestine that have not yet been stimulated to further development become tightly coiled when removed from the host and placed in a physiological salt solution, but those which have been stimulated to development apparently lose the power of becoming tightly coiled under similar conditions.

(4) Larvæ which have been stimulated to further development in the intestine will molt even after being removed from that organ. The molting process may be hastened by high temperatures and suppressed by low temperatures.

(5) Larvæ which have not yet been stimulated to further development in the small intestine can not be caused to molt by a high temperature.

(6) With the beginning of development in the small intestine the larvæ lose the power of surviving for considerable lengths of time outside of the host. They afterward become more persistent, however, in direct proportion to their increasing age.

(7) When removed from the host within 24 hours after artificial infection intestinal trichinæ often undergo spontaneous disintegration which may be due to the sudden change of environment, lack of food, or possibly the liberation of toxic substances which affect the parasites while in an artificial medium.

(8) Larvæ which molt after removal from the host have been observed occasionally to decrease in size. It is suggested that the dwarfed condition is possibly due to lack of food.

(9) After the first and subsequent molts the tolerance of the larvæ to various toxic agents is replaced by a marked sensitiveness to such agents which decreases, however, with advancing age.

(10) Under the influence of potassium cyanid the worms undergo disintegration and exhibit susceptibility to the poison along the major axis which in the growing forms appears to be greatest in regions where growth takes place most rapidly.

(11) Modifications in the permeability of the cuticle do not appear to be directly responsible for the changes in susceptibility. The changes probably result from a reorganization of the protoplasm coincident with growth, differentiation, and age.

(12) Attempts to induce molting in the larvæ which have been decapsuled by artificial digestion and afterwards kept *in vitro* under various conditions have thus far failed to yield successful results.

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